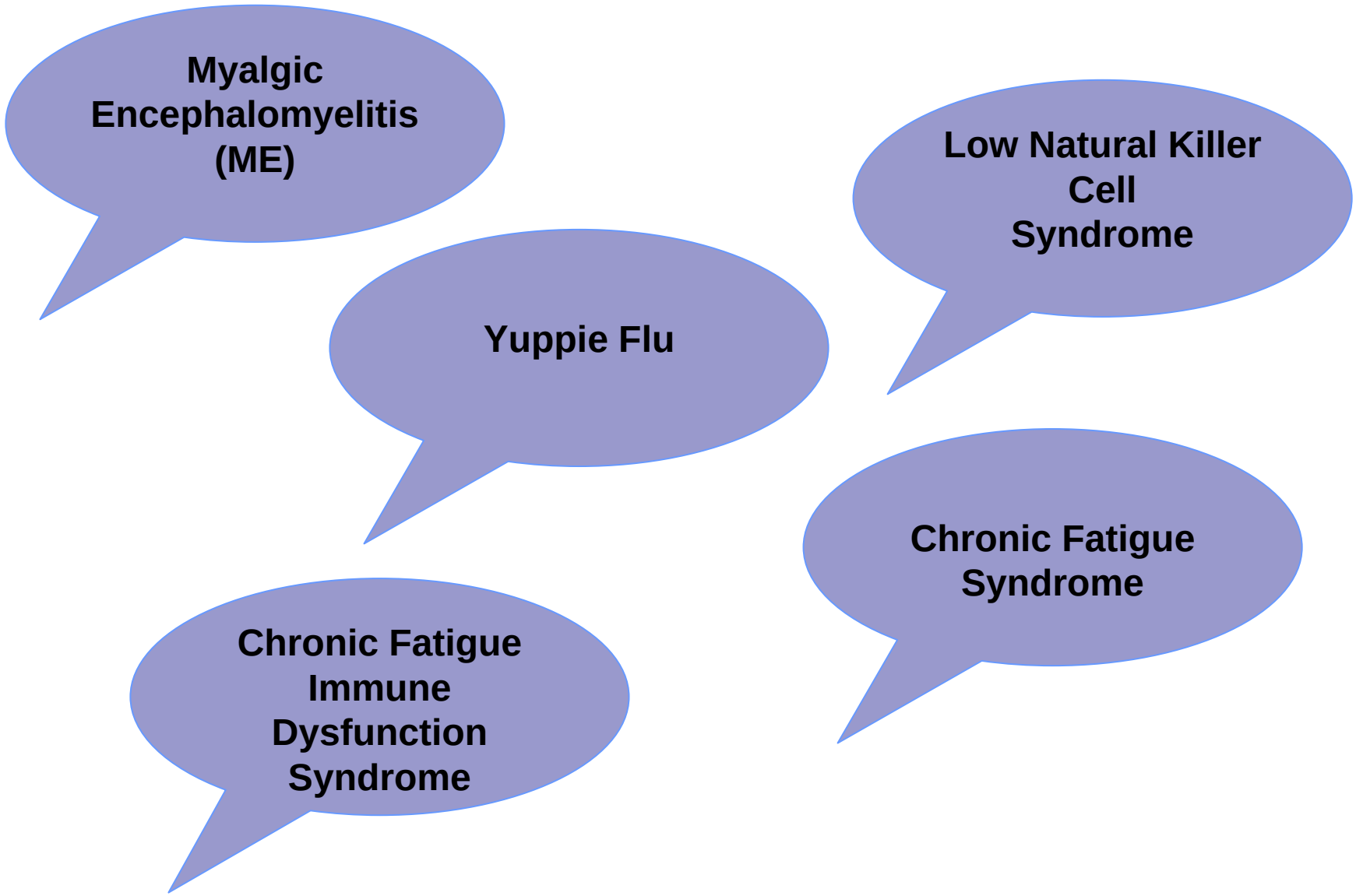
CFS / ME Symposium
Malmo, Sweden

Daniel L. Peterson, MD
Incline Village, Nevada, USA

October 17, 2007

Illness of Many Names



Myth #1:

ME/CFS is a relatively rare disorder

Facts:

- Prevalence in U.S.

  200-400 per 100,000 people

- Number of people affected in U.S.

  500,000 people

- Number of people affected in Sweden

  16,000 – 32,000 people

Annual Economic Loss

$9 billion per year in the U.S.

$2.1 billion Swedish Kronor In Sweden

Prevalence of CFS in the US

- CDC Estimated Prevalence
  → 235/100,000 = over 1 million patients (2006)

- Market potential of a diagnostic marker test
  → $120,000,000/yr

- Market potential of effective therapy
  → $250,000,000/yr

Myth #2:

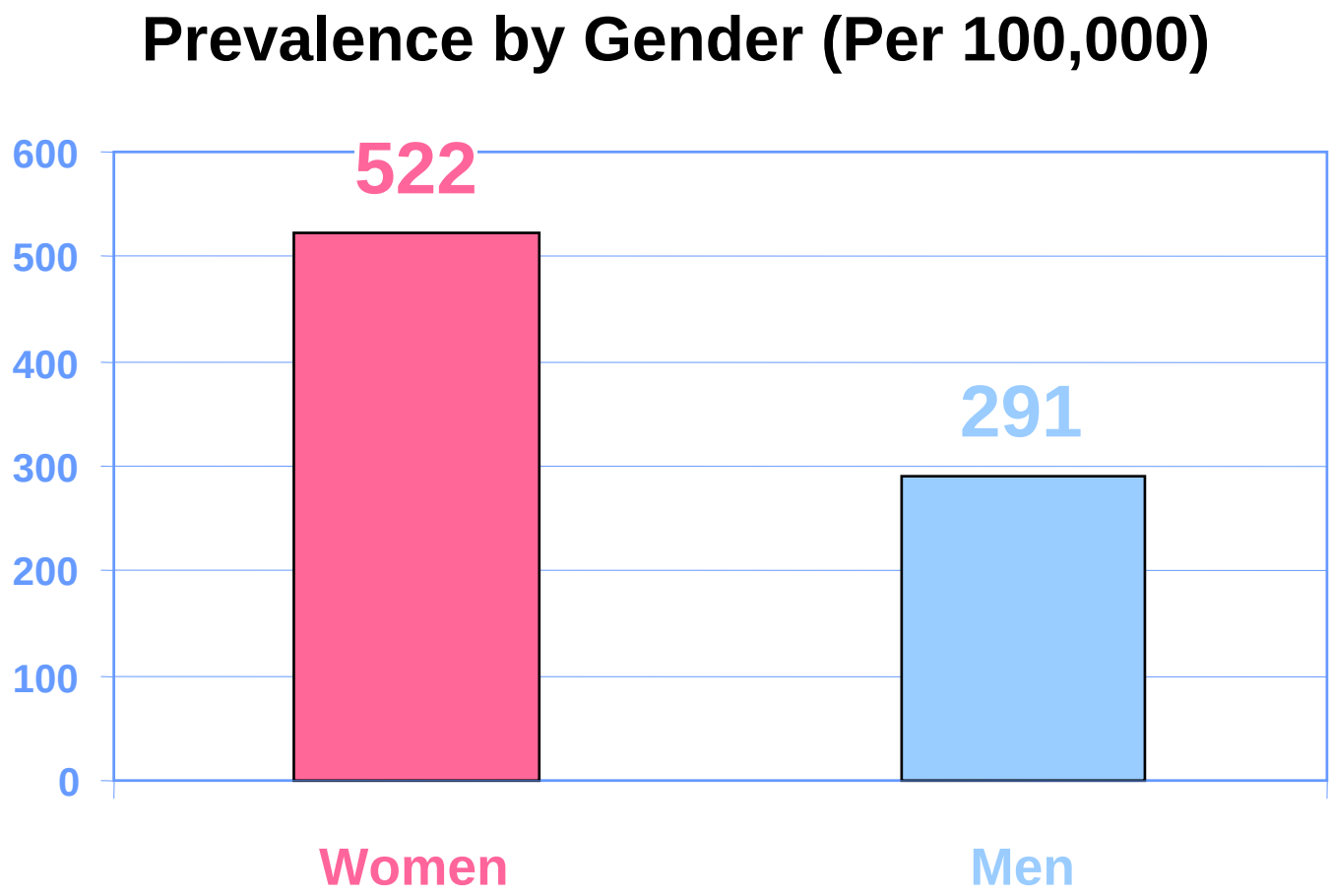
The highest prevalence is among young, affluent, white professionals

Fact:

Women, men and children from all socio-economic backgrounds are affected by CFS

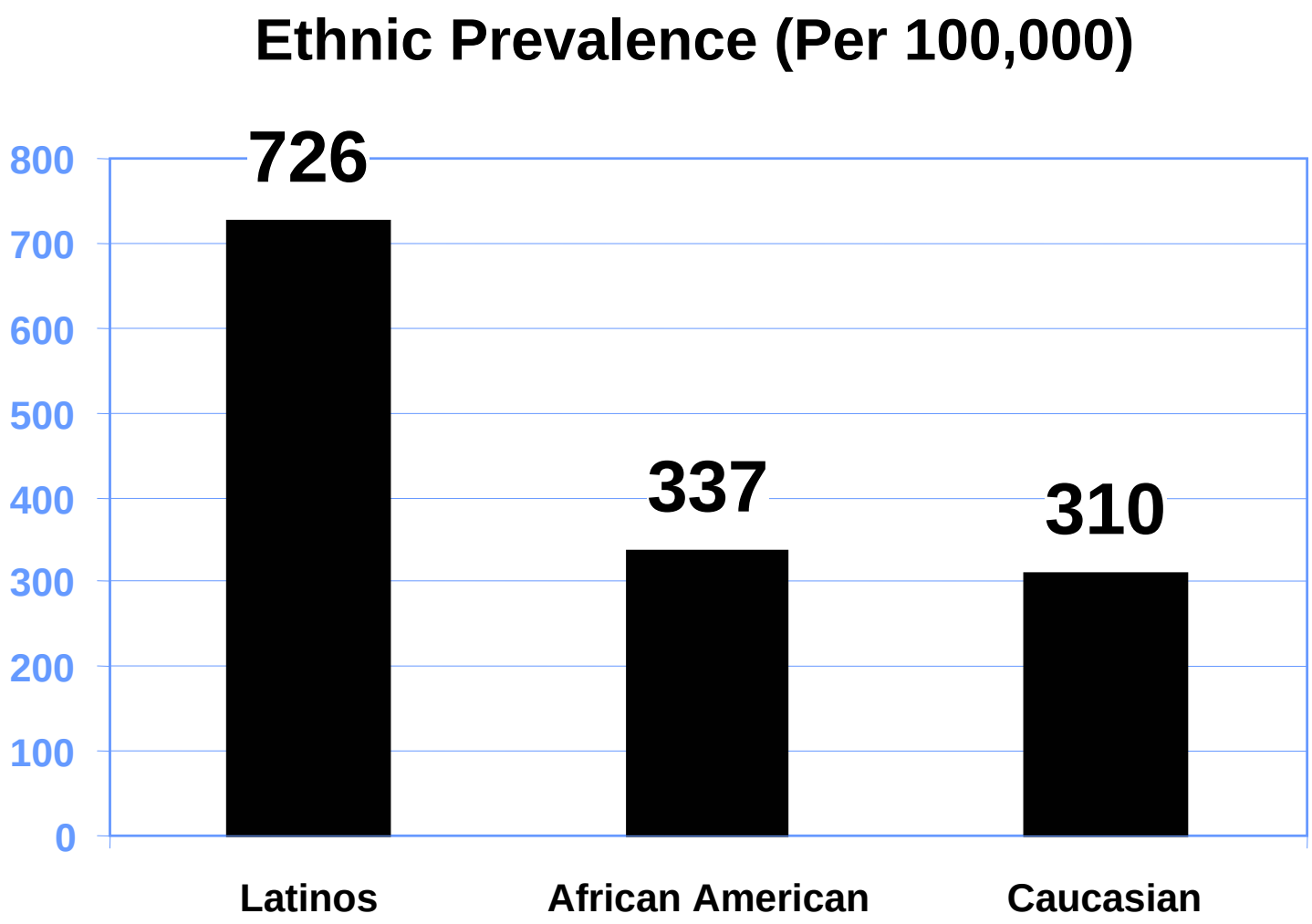
Fact: Gender

Women have a much higher rate of CFS than men



Fact: Race

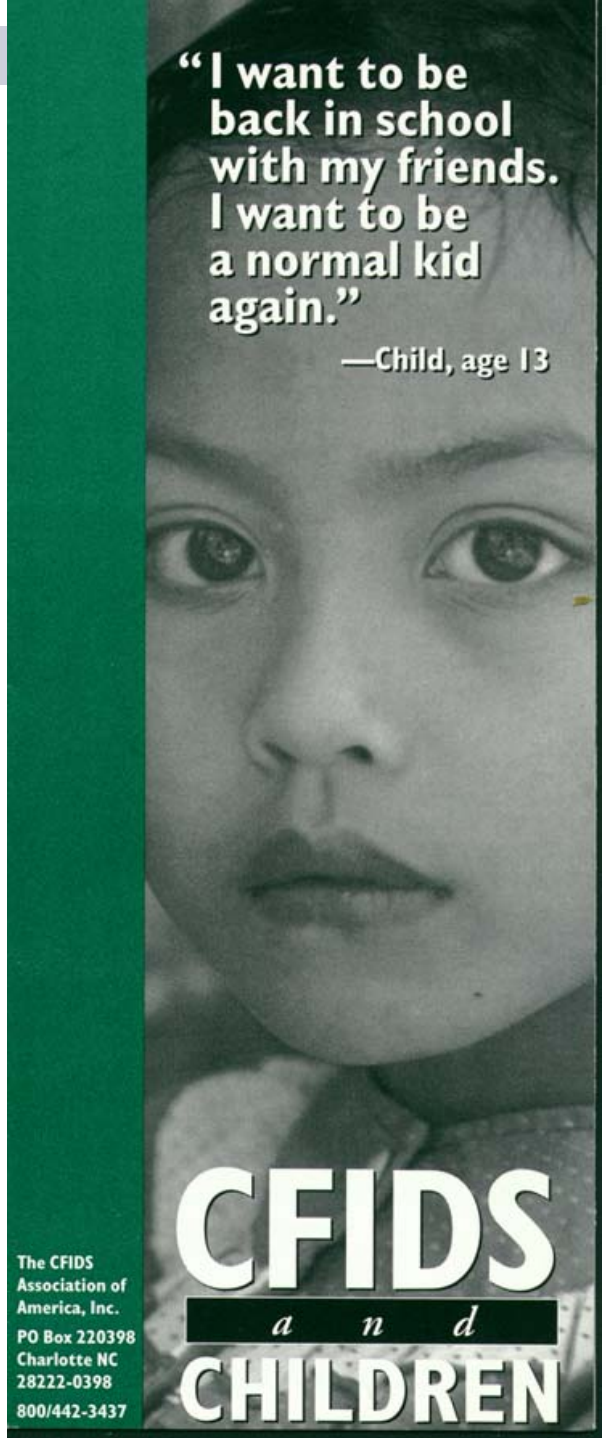
Latinos have highest prevalence of CFS



Overlooked Population

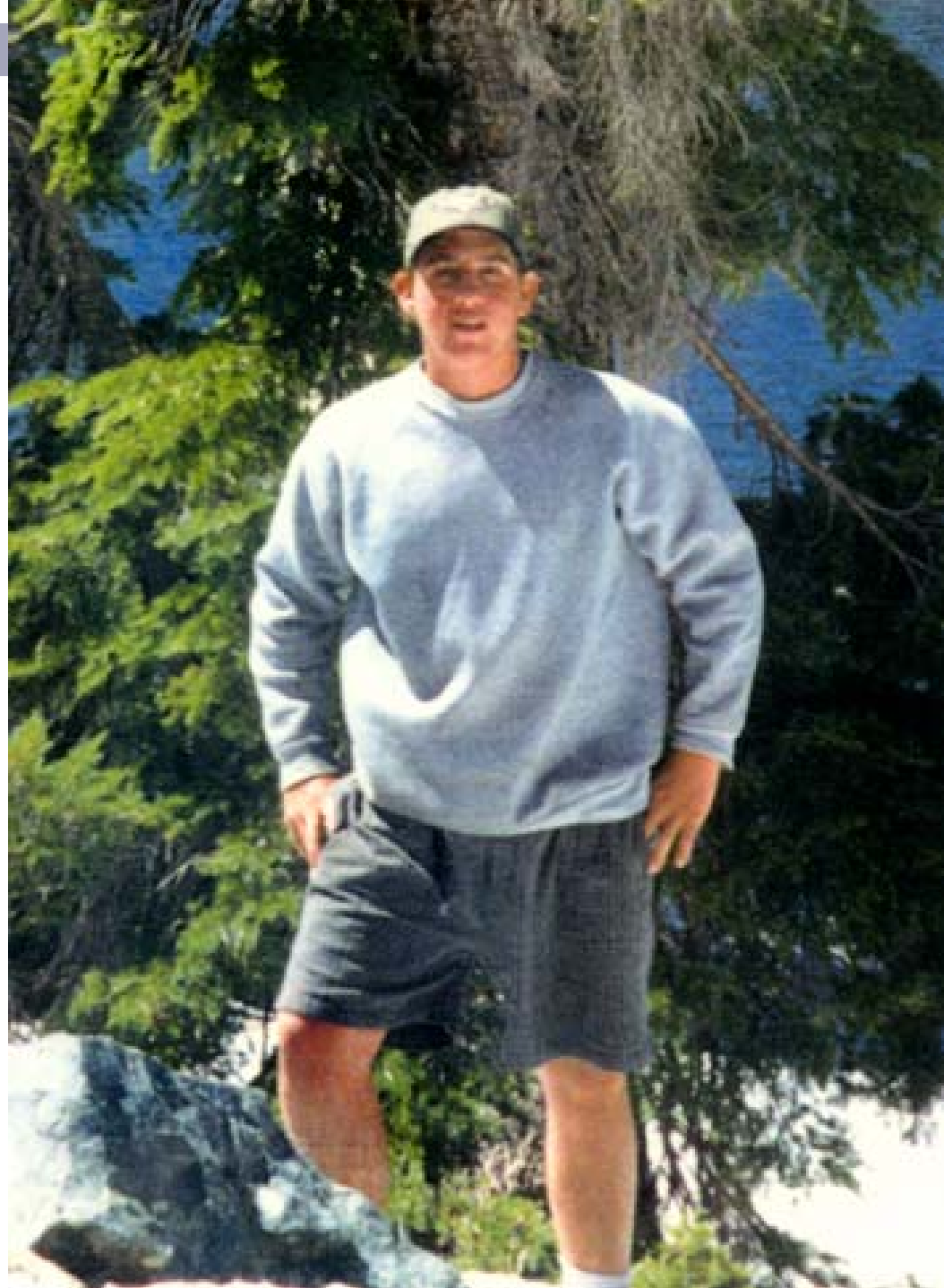
Fact:

Children with CFS are under-recognized and under-studied.



Pediatric CFS

- Has significant impact on educational achievement
- May result in significant health identity confusion and prolonged disability



David Bell, M.D.
Lyndonville Pediatric Study
CFSCC Meeting, April 1998

Myth #3

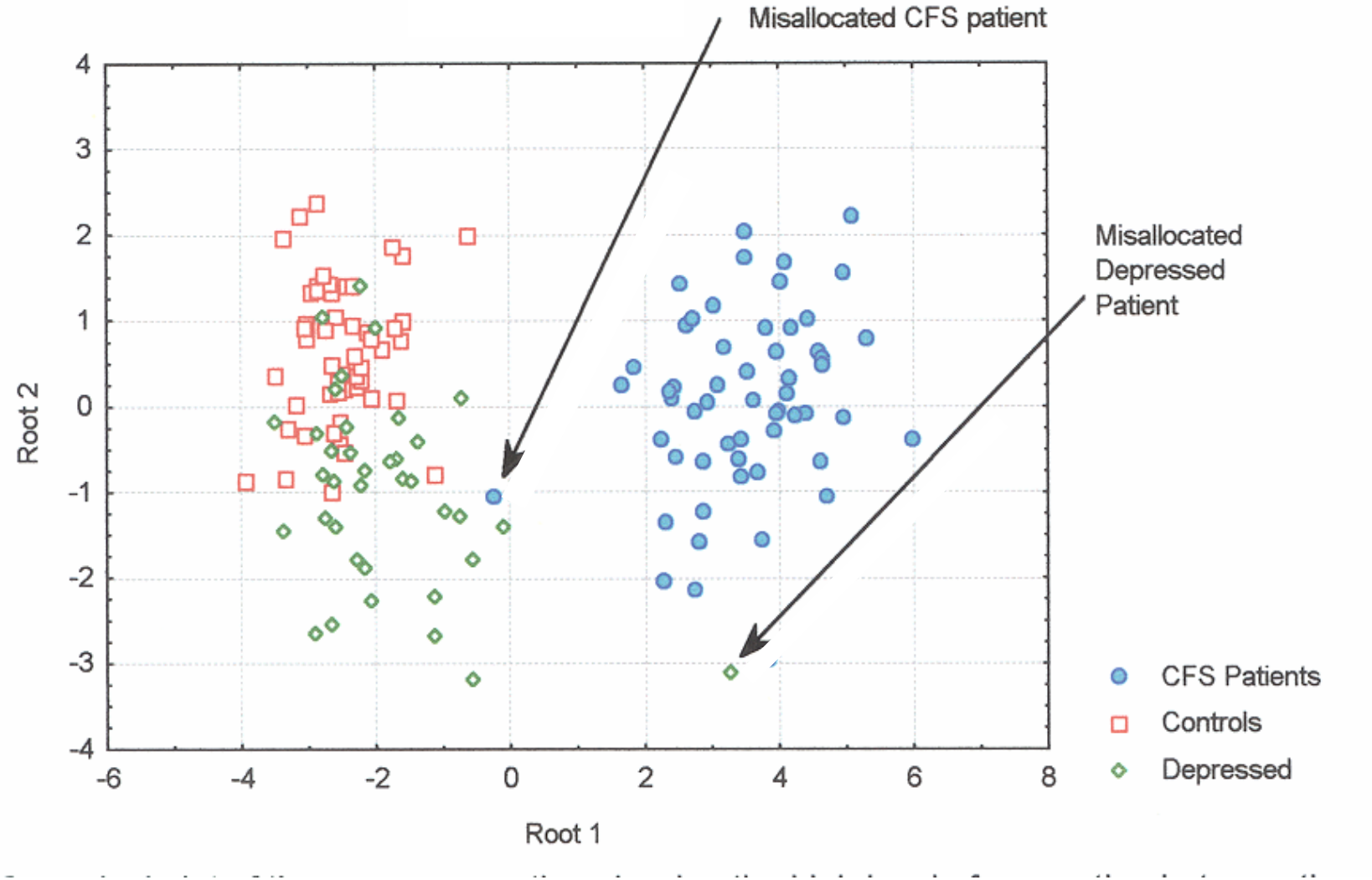
CFS is a form of depression

Fact:

CFS differs from depression in:

- Onset
- Incidence
- Major symptoms
- Attributions
- Lab tests
- Responses to treatment
- Rehabilitation
- Gene expression

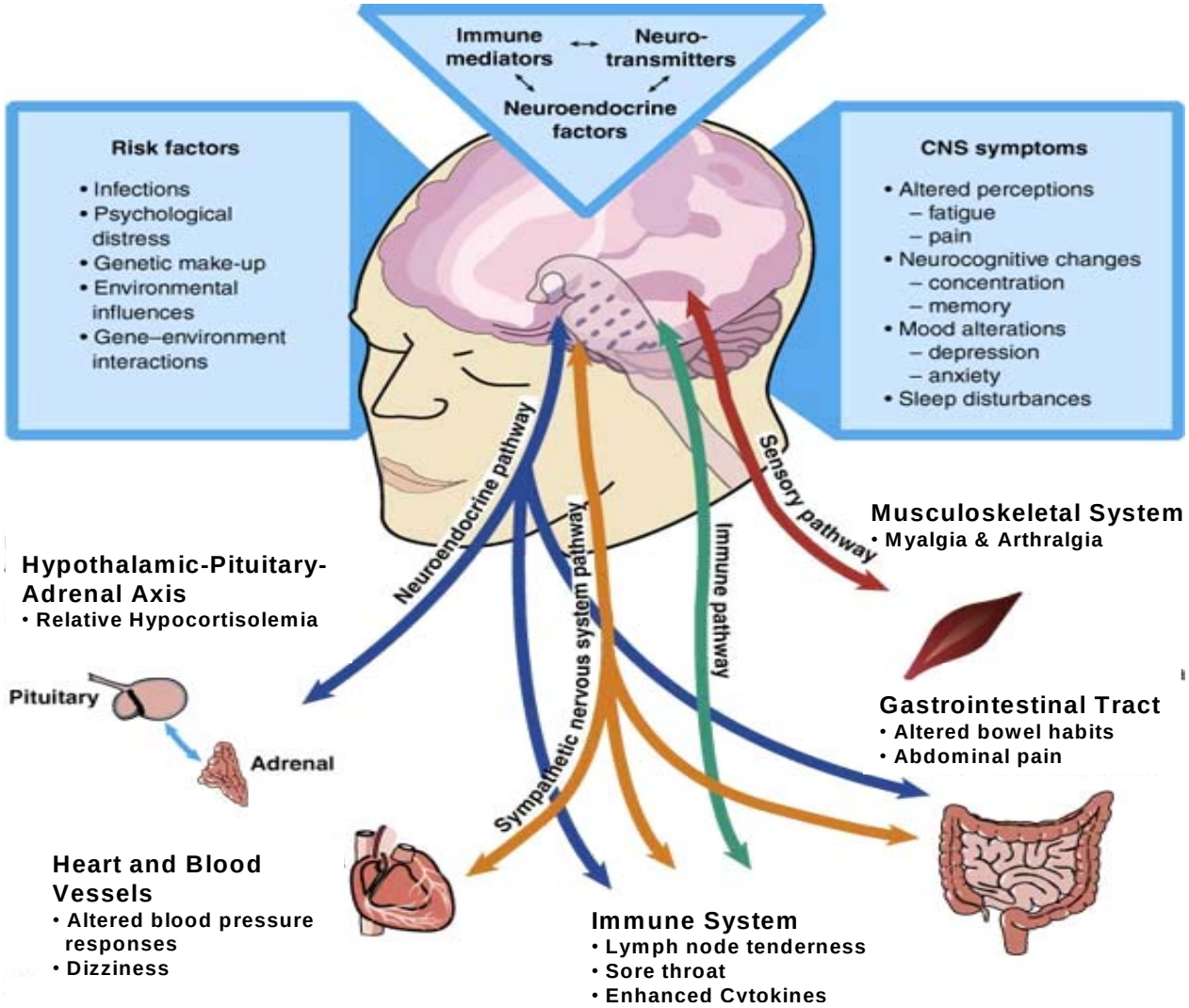
CFS is distinct from Healthy and Depressed Controls



Common symptoms

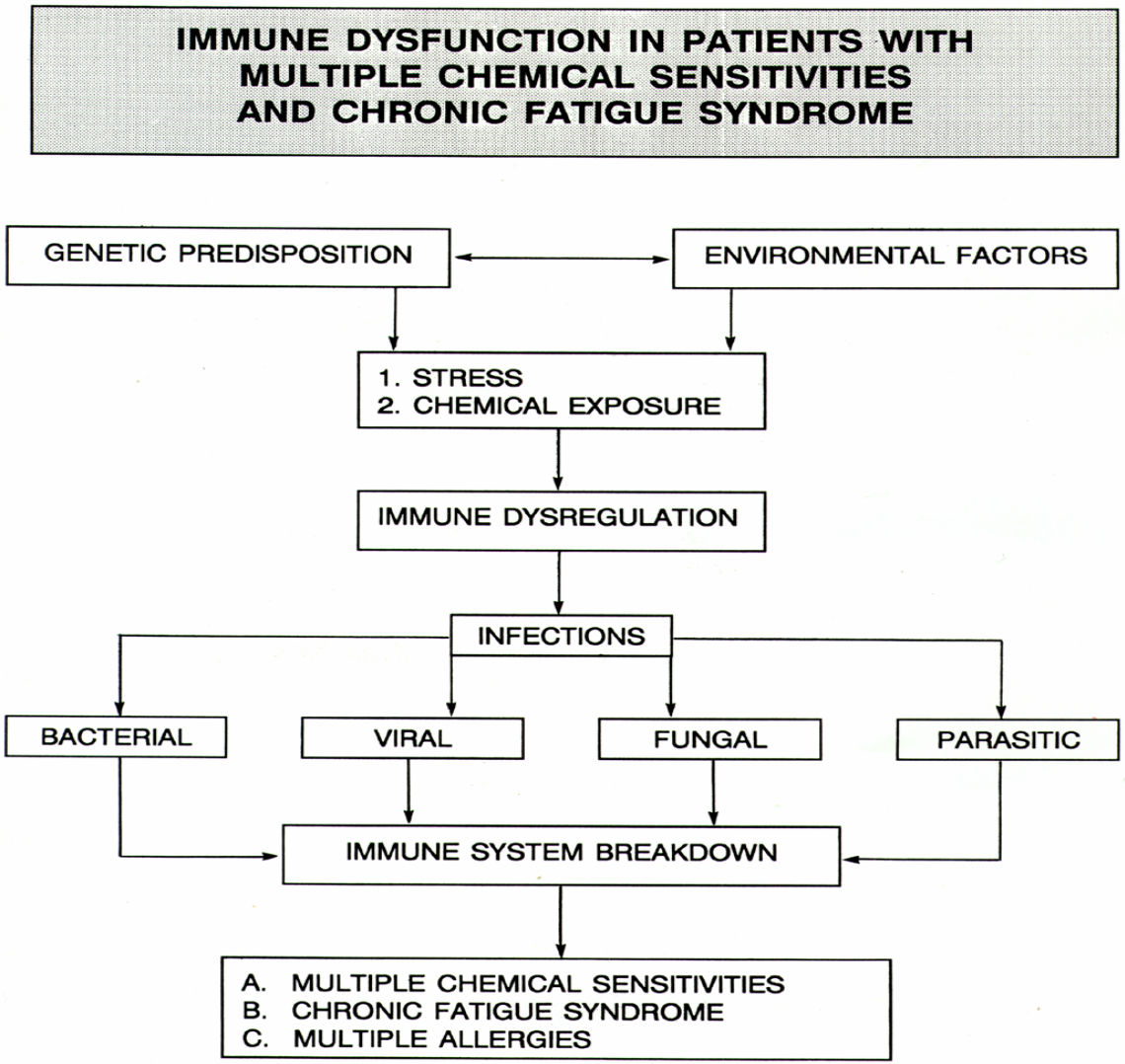
Symptom	Frequency (%)	Symptom	Frequency (%)
Fatigue	100	Stiffness	50-60
Low-grade fever	60-95	Arthralgia	40-50
Myalgias	20-95	Tachycardia	40-50
Sleep disorders	15-90	Dizziness	30-50
Depression	70-85	Anorexia	30-40
Impaired cognition	50-85	Cough	30-40
Headaches	35-85	Dry eyes	30-40
Sore throat	50-75	Dry mouth	30-40
Anxiety	50-70	Finger swelling	30-40
Muscle weakness	40-70	Night sweats	30-40
Blurred vision	50-60	Painful lymph nodes	30-40
Nausea	50-60	Parasthesias	30-40
Post-exertional malaise	50-60	Rash	30-40

Fact: Multiple systems are involved in ME/CFS



Source: Royal Australian College of Physicians

Proposed Model



CDC Criteria (Fakuda 1994)

- Persistent or relapsing fatigue of 6 months or longer in duration
- Other known medical conditions excluded by clinical diagnosis
- Concurrently have the following symptoms:

✓ Impaired memory or concentration
✓ Sore throat
✓ Tender cervical or axillary lymph nodes
✓ Muscle pain
✓ Multi-joint pain
✓ New headaches
✓ Unrefreshing sleep
✓ Post exertional malaise lasting more than 24 hours

CDC definition of Chronic Fatigue (Fakuda 1994

① Clinically unexplained persistent or relapsing fatigue of new or definite onset which is:

- Not the result of ongoing exertion
- Not alleviated by rest
- Causes substantial reduction in occupational, educational, social or personal activities

- Annals of Internal Medicine, 1994

CDC definition of Chronic Fatigue (Fakuda 1994

② Concurrent occurrence of 4 or more of the following symptoms in at least 6 consecutive months of illness (but not predating illness):

- Impairment in short-term memory or concentration
- Sore throat
- Tenderness in cervical or axillary lymph nodes
- Multi-joint pain without swelling or redness
- Headaches or a new type, pattern or severity
- Non-refreshing sleep
- Post-exertional malaise lasting more than 24 hours

- Annals of Internal Medicine, 1994

Canadian Consensus Document (Carruthers 2003)

Myalgic Encephalomyelitis / Chronic Fatigue Syndrome: Clinical Working Case Definition, Diagnostic and Treatment Protocols

Journal of Chronic Fatigue Syndrome
Vol. 11(1) 2003

Clinical Working Case Definition

A patient with ME/CFS will meet

the criteria for:

Fatigue
Post-exertional malaise and/ or fatigue
Sleep dysfunction
Pain
Neurological/cognitive manifestations
(two or more)
At least one symptom from two of the following categories

a. Autonomic manifestations
b. Neuroendocrine manifestations
c. Immune manifestations

Illness that persists for at least six
months and has a distinct onset
(although onset may have been gradual)

Neurological/Cognitive Manifestations

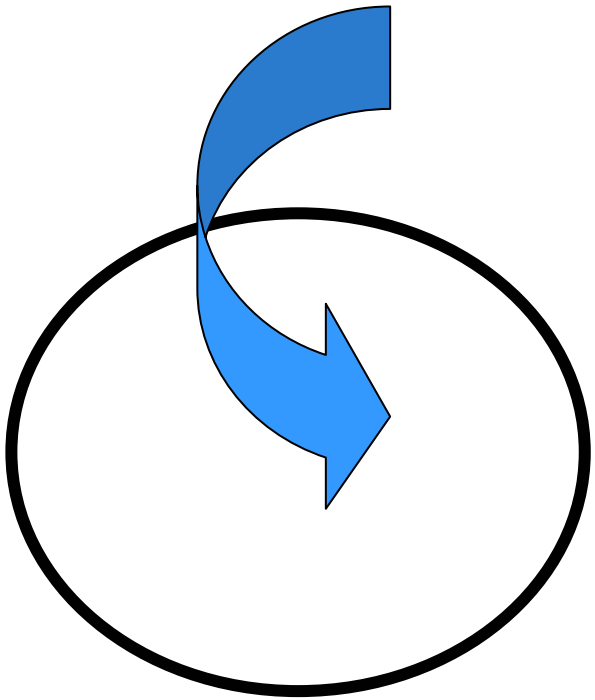
Two or more of the following:

- Confusion
- Impairment of concentration and short-term memory consolidation
- Difficulty with information processing, categorizing, and word retrieval
- Disorientation
- Perceptual and sensory disturbances
- Ataxia
- Muscle weakness
- Fasciculations
- Overload phenomena: cognitive, sensory, and emotional

Major difference between CDC and Canadian Consensus

- Neurocognitive manifestations
- Easier for practicing physicians

Inclusion



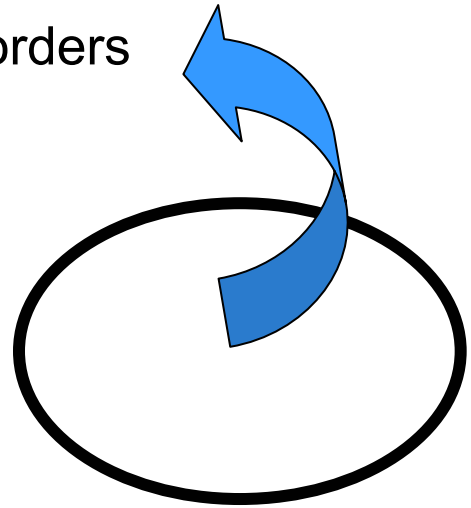
- Symptoms must have begun or have been significantly altered after onset
  - Unlikely that a patient will suffer from all symptoms in criteria 5 and 6
  - Disturbances tend to form symptom clusters that may fluctuate and change over time
  - Children often have numerous prominent symptoms but their order of severity tends to vary from day to day

- Active disease processes that explain most of the major symptoms

Exclusions

- Addison's disease
- Cushing's Syndrome
- Hypothyroidism
- Hyperthyroidism
- Iron deficiency
- Other treatable forms of anemia
- Iron overload syndrome

- Diabetes mellitus
- Cancer
- Treatable sleep disorders
- Rheumatological disorders
- Immune disorders
- Infectious diseases
- Primary psychiatric disorders
- Substance abuse

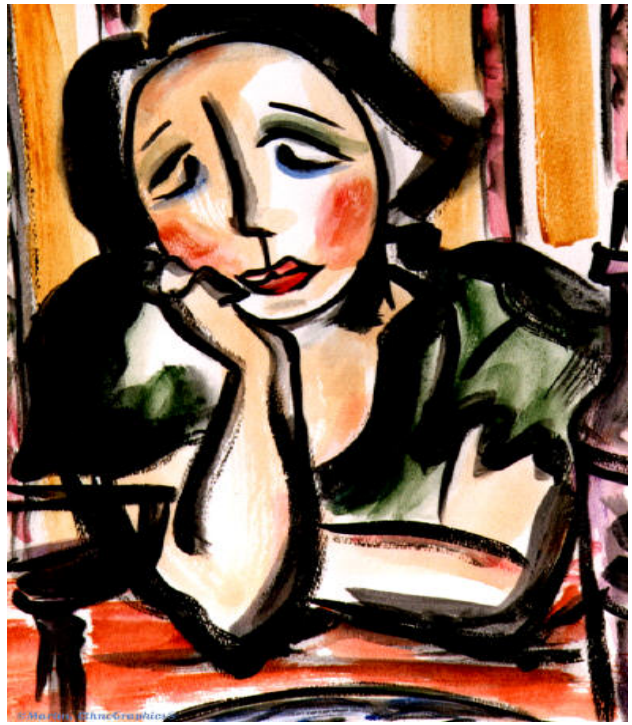


Exclusion of other diagnoses, which cannot be reasonably excluded by the patient's history and physical exam, is achieved by laboratory testing and imaging

If a potentially confounding medical condition is under control, the diagnosis of ME/CFS can be entertained if patients meet the criteria otherwise.

1. Fatigue

- Significant degree of physical and mental fatigue
  - New onset
  - Unexplained
  - Persistent
  - Recurrent
- Fatigue substantially reduces activity level



2. Post-Exertional Malaise and/or Fatigue

- Inappropriate loss of physical and mental stamina
- Rapid muscular and cognitive fatigability
- Post exertional malaise and/or fatigue and/or pain
- Tendency for other association symptoms to worsen
- Pathologically slow recovery period – usually 24 hours or longer

3. Sleep Dysfunction

- Unrefreshed sleep, or
- Sleep quantity, or
- Rhythm disturbances, such as reversed or chaotic diurnal sleep rhythms

A small number of patients have no sleep dysfunction, but no other diagnosis fits except ME/CFS

4. Pain

- Significant degree of myalgia
- Pain in the muscles and/or joints, often widespread and migratory in nature
- Significant headaches of new type, pattern, or severity

A small number of patients have no pain, but no other diagnosis fits except ME/CFS

5. Other Symptoms

Autonomic Manifestations

- Orthostatic intolerance-neurally mediated hypotension (NMH)
- Delayed postural hypotension
- Light-headedness
- Extreme pallor
- Nausea and irritable bowel syndrome
- Urinary frequency and bladder dysfunction
- Palpitations with or without cardiac arrhythmias
- Exertional dyspnea

Neuroendocrine Manifestations

- Loss of thermostatic stability
- Marked weight change
- Loss of adaptability and worsening of symptoms with stress

Immune Manifestations

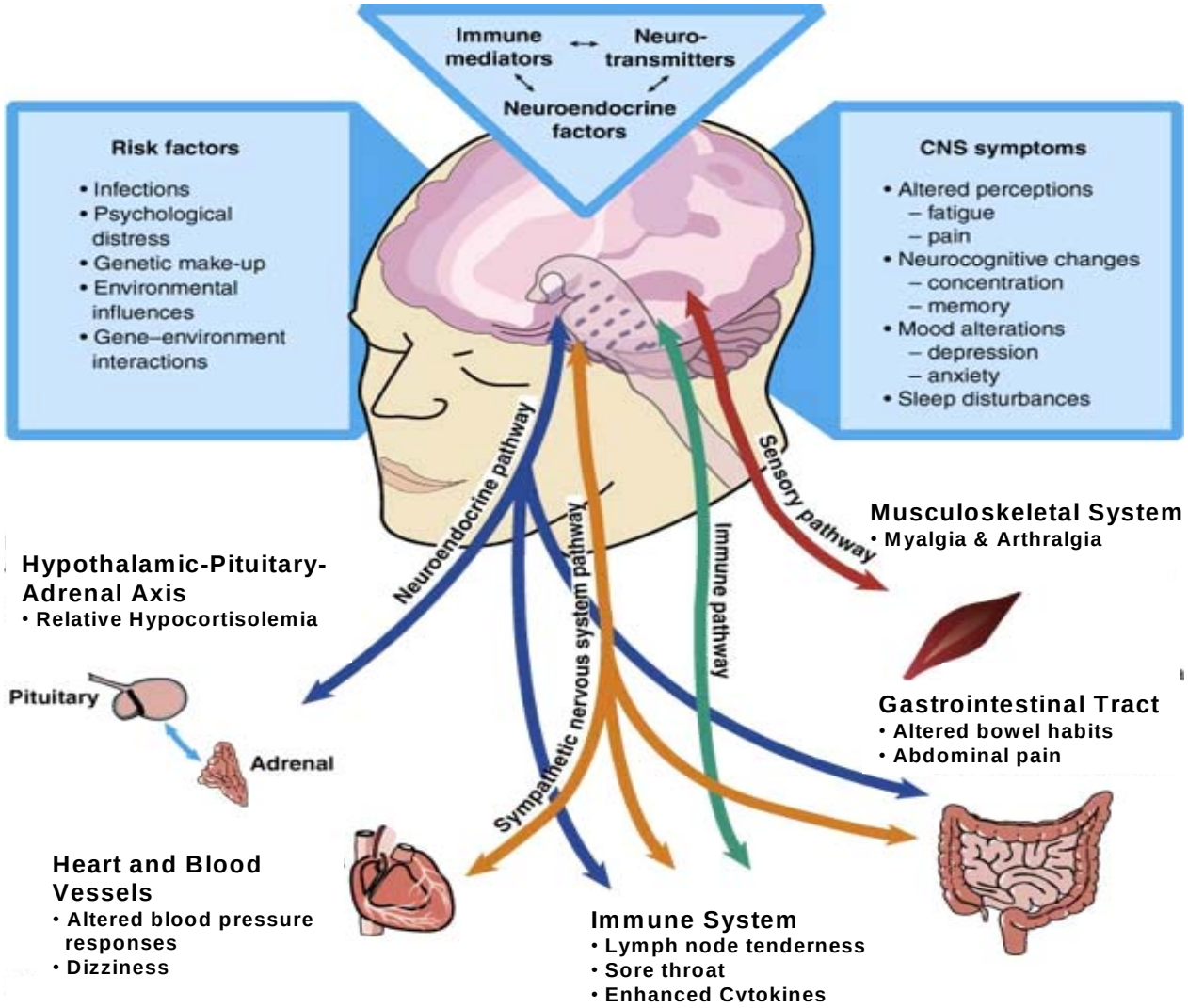
- Tender lymph nodes
- Recurrent sore throat
- Recurrent flu-like symptoms
- General malaise
- New sensitivities to food, medications, and/or chemicals

6. Illness Duration

- Illness persists for at least 6 months
- Usually a distinct onset, although it may be gradual
- (Preliminary diagnosis may be possible earlier than 6 months)
- (Illness duration of 3 months is appropriate for children)

Some patients may have been unhealthy for other reasons prior to onset of ME/CFS and lack detectable triggers, and/or have more gradual or insidious onset

Multiple systems involved in CFS



Source: Royal Australasian College of Physicians

Immunological Profiling in ME/CFS

ME/CFS patients frequently demonstrate:

- Low Natural Killer cell activity
- Activation of 2-5A synthetase antiviral pathway
- Altered form of RNaseL
- Viral infections *

PAST RESEARCH:

Infectious Agents Associated with ME/ CFS

- **Background**

- Infectious agents may trigger and perpetuate CFS
- Pathogens associated with CFS have in common that they are difficult or impossible for the immune system to eliminate.
- Human herpes viruses associated with CFS include:

  HHV4 (EBV), HHV6 and HHV7
- Other associated infectious agents include: paravovirus B19, enteroviruses, Borrelia burgdorferi, and mycoplasma fermentans

Current Research:

- Patient Sample Repository and Database
- Gene Expression Profiling on ME/CFS
- Virus Expression Profiling in ME/CFS
- Cytokine and Proteomic Profiles by Antibody Array in ME/CFS

Sample Repository

A large ME/CFS sample repository containing:

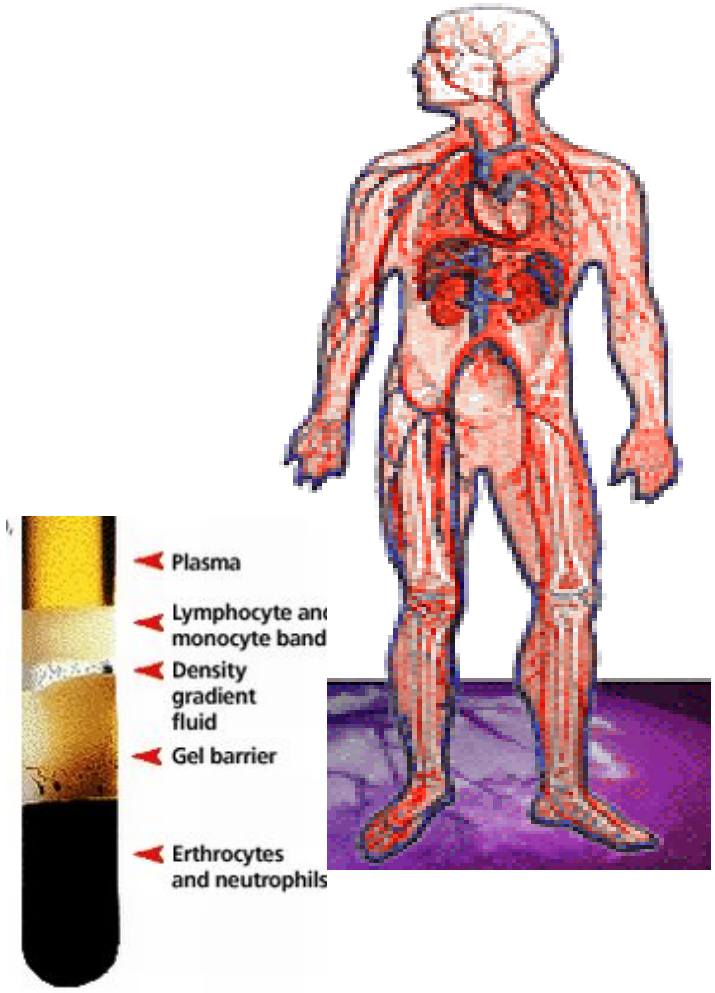
- More than 5000 samples
- Fully categorized, searchable database of patient and sample data
- 25,000 sample capacity cryo-storage freezer
- -70°C and -150°C freezers
- Bio-processing lab for sample preparation

Repository for Worldwide Research

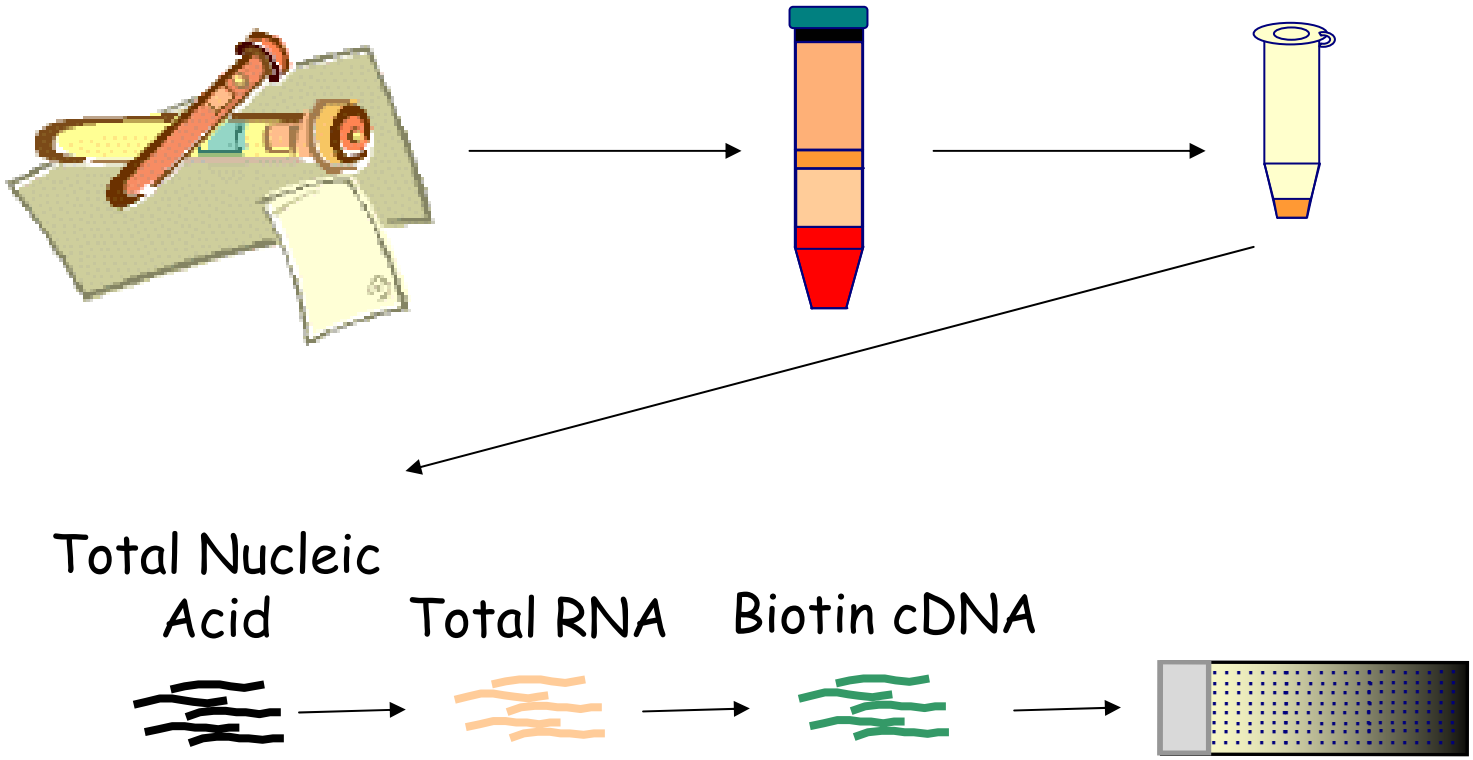
- Collaborations have been established and samples sent worldwide to investigators using the state of the art technologies such as:
  - Gene expression profiling
  - Virus expression profiling
  - Epigenetic profiling
  - Inflammatory mediator expression

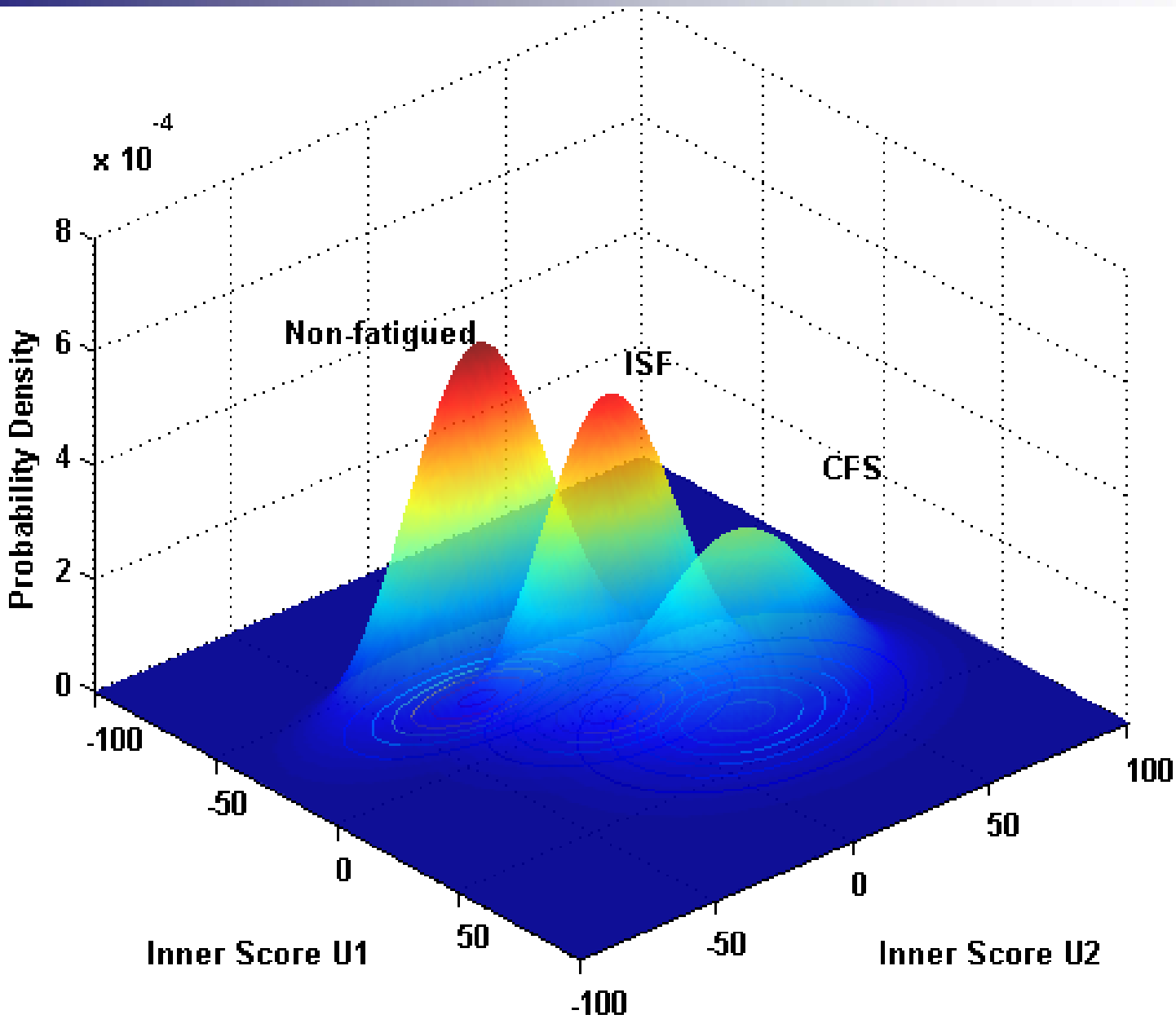
What sample can be used for gene and virus expression profiling?

- No lesion
- Peripheral blood
  - 5 liters of blood circulating
  - Blood cells can serve as sentinels of disease processes
  - Plasma has proteins from throughout the body
- Information rich sample!



Measuring Gene and Virus Expression





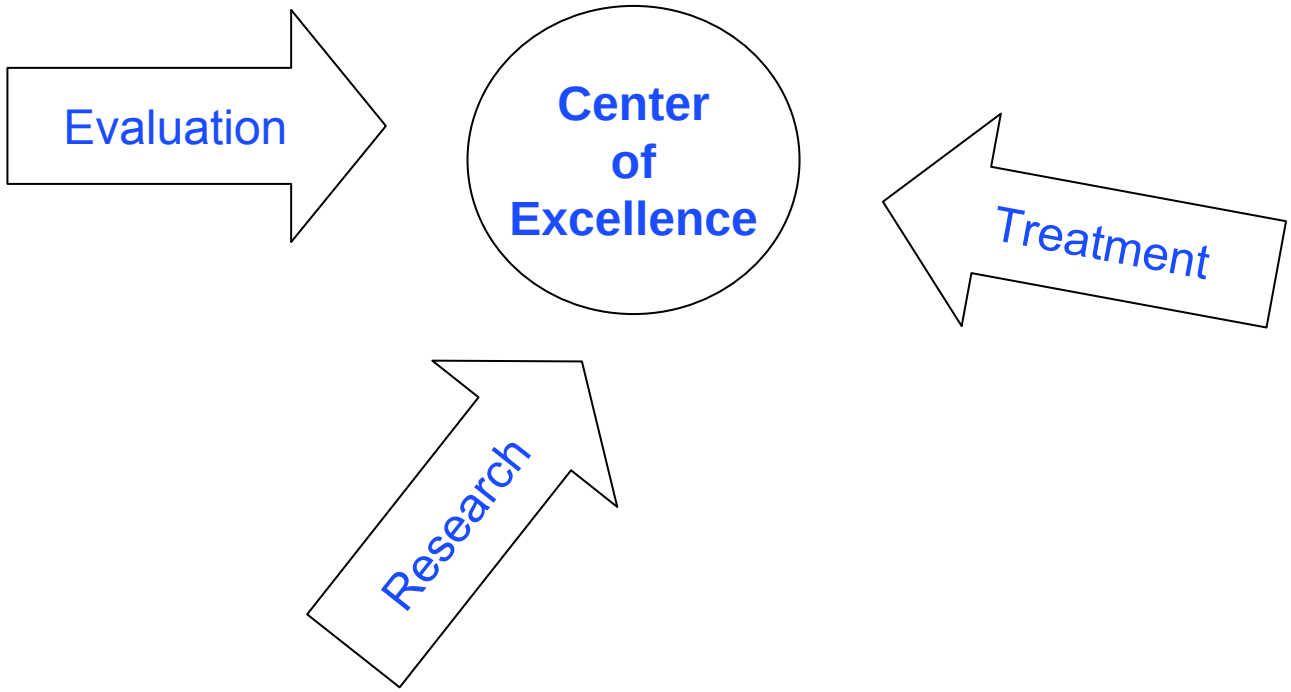
Broderick et al, Pharmacogenomics 2006

Virus Expression Microarray Results

- Many viruses are expressed in healthy controls verifying the host response to infection is key to disease development
  - Several viruses were found to be differentially expressed in both UK and US studies!
  - Patterns of virus expression differentiates people with CFS from controls

Neuroimmune Institute Mission

Comprehensive Evaluation, Treatment, and Research



Current Research:

- Gene Expression Profiling on ME/CFS
- Virus Expression Profiling in CFS
- Clonal T cell Receptor Gamma Rearrangements and MCL Development
- Chromosomally integrated HHV6 (CIHHV6)
- Cytokine and Proteomic Profiles by Antibody Array

Identification of Mammalian viruses expressed in a cohort of CFS patients with clonal TCRγ (cTCRG) rearrangements and Mantle Cell Lymphoma (MCL)

Cancer and Inflammation Program and Lab of Molecular Technology, Center for Cancer Research National Cancer Institute (NCI)- Frederick, MD, USA

Francis W. Ruscetti. PhD

Ying Huang,

Cassio Baptista, PhD

Sierra Internal Medicine and Simaron, LLC Incline Village, NV, USA

Darren White

Judy A. Mikovits, PhD

Vincent C. Lombardi, PhD

Significant Cytokine Differences between CFS/MCL+and CFS/MCL-				
Cytokine pg/mL	CFS/MCL+	CFS/MCL-	Significance	function
IL-7	50/8	<15.0	.0449	B cell development
IFN-α	65.1	<15.0	.0302	Antiviral response
IL-6	2044.7	11.7	.0025	Inflammation
MIP1-α	4205.7	38.2	.0378	
IP-10	572.6	31.4	.0039	Interferon response
TNF-α	65.1	<15.0	.0302	inflammation
MCP-1	589.0	286.5	.0407	

MPSS Overview

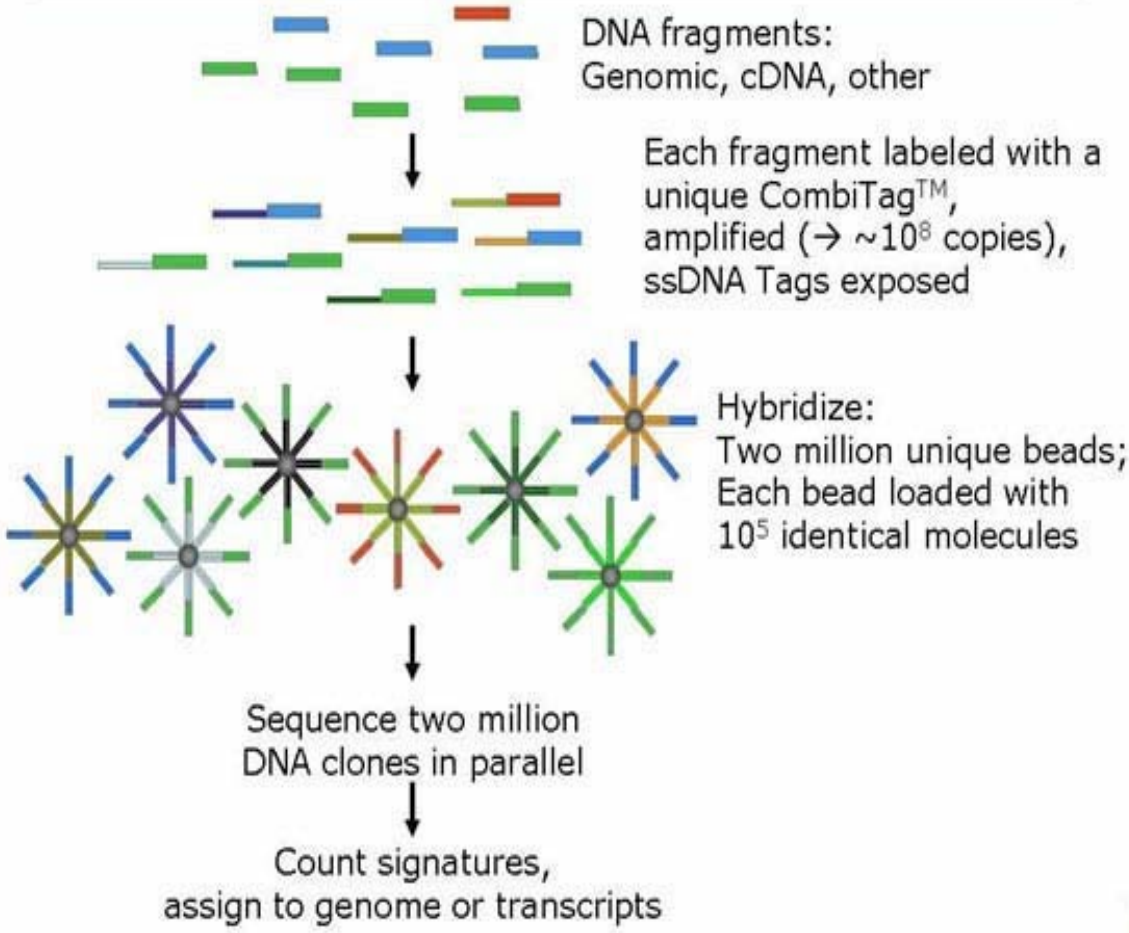


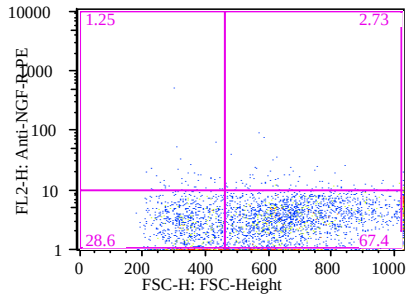
Table 1. Top thirteen signature sequences having significantly different expression levels between CFS and Normal groups in A European Cohort, with BLAST hits from the virus database.

Genbank Accession	Virus genome	TPM CFS	TPM Normal	P value
NC_005261.1	Bovine herpesvirus 5, complete genome	342	128	1.14E-08
NC_001847.1	Bovine herpesvirus 1, complete genome	342	128	1.14E-08
NC_003038.1	Invertebrate iridescent virus 6, complete genome	71	0	7.32E-07
NC_004161.1	Reston Ebola virus, complete genome	5	98	1.64E-06
NC_006144.1	Simian adenovirus A, complete genome	575	349	2.95E-05
NC_002052.1	Tomato spotted wilt virus RNA L, complete genome	54	0	1.52E-05
NC_005337.1	Bovine papular stomatitis virus, complete genome	269	481	2.37E-05
NC_001731.1	Molluscum contagiosum virus, complete genome	269	481	2.37E-05
NC_004197.1	Bovine papillomavirus - 3, complete genome	926	643	3.90E-05
NC_004451.1	Simian picornavirus 1, complete genome	161	59	9.38E-05
NC_005990.1	Chuzan virus, complete genome	0	50	1.37E-04
NC_006146.1	Cercopithecine herpesvirus 15, complete genome	42	0	1.80E-04
NC_006560.1	Cercopithecine herpesvirus 2, complete genome	0	43	3.41E-04

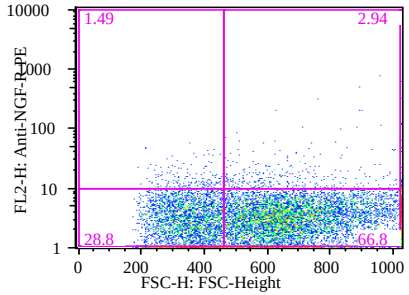
Table 2. MPSS signature sequences and custom microarray probe sequences with significantly different expression levels between CFS and Normal groups, which overlapped between European (using MPSS) and American (using custom microarray) studies.

Virus genome	Genbank Accession	MPSS TPM CFS	MPSS TPM Normal	P value
Pestivirus Giraffe-1, complete genome	NC_003678.1	24	0	4.22E-03
Simian picornavirus 1, **complete** genome	NC_004451.1	161	59	9.38E-05
Bovine herpesvirus 5, complete genome	NC_005261.1	342	128	1.14E-08
Bovine herpesvirus 1, complete genome	NC_001847.1	342	128	1.14E-08
Bovine herpesvirus 4, complete genome	NC_002665.1	35	12	4.00E-02
Cercopithecine herpesvirus 15, complete genome	NC_006146.1	42	0	1.80E-04
Bovine papillomavirus - 3, complete genome	NC_004197.1	926	643	3.90E-05
Simian T-lymphotropic virus 3, complete genome	NC_003323.1	48	10	5.68E-03

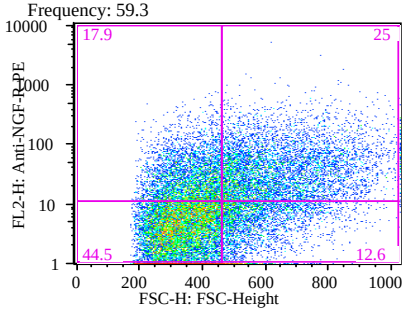
Figure 2. FACS analysis of antibody staining to NGF (y axis) and side scatter X axis. The controls are shown in the top two panels and four individual patients are shown in the lower four panels. Populations of cells in the top two quadrants are transduced (expressing NGFR) and viable.The % transduction in the four patient samples ranged from 43-57 and 90% viable.



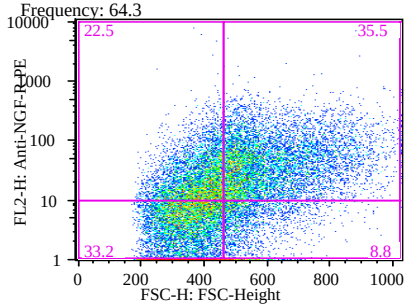
adultnorm.289
Pseudo-color graph (unsmoothed, high res)
Event Count: 3192
Frequency: 59.3



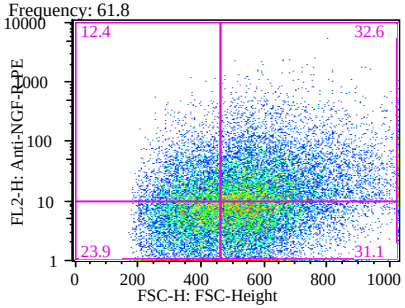
adultnorm.290
Pseudo-color graph (unsmoothed, high res)
Event Count: 11442
Frequency: 64.3



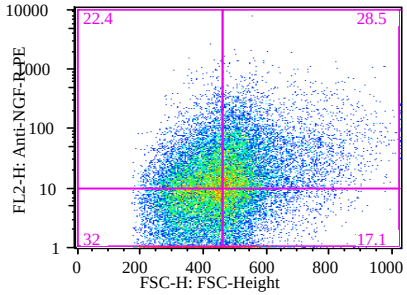
26gk.294
Pseudo-color graph (unsmoothed, high res)
Event Count: 26276
Frequency: 61.8



27gk.296
Pseudo-color graph (unsmoothed, high res)
Event Count: 24809
Frequency: 63.3



26jk.293
Pseudo-color graph (unsmoothed, high res)
Event Count: 26972
Frequency: 81.4



27jk.297
Pseudo-color graph (unsmoothed, high res)
Event Count: 27089
Frequency: 82.5

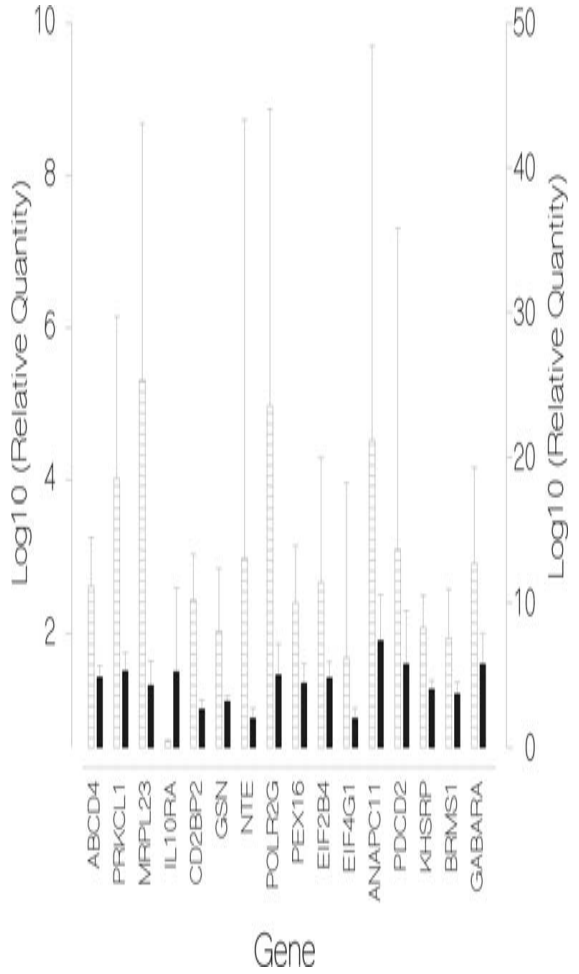


Figure 3. Bar chart showing the mean relative quantity of mRNA transcripts in test (shaded) and control (solid black) groups for 16 genes that were differentially expressed in CFS in our pilot study. Error bars indicate the standard deviation from the mean in each case. All values for the mean relative quantity mRNA transcript are shown on the left y axis, except those for NTE and EIF4G1, which are shown on the right y axis (from Kaushik, Fear et al. 2005).

Figure 4. Relative quantities of the EBI2 gene in CFS patients (on the right) and in normal blood donors (on the left); 11 of 20 CFS patients in this analysis showed levels significantly raised compared with the normal controls. CFS patients as a group had a 3.44 fold higher EBI2 mean level compared with normal controls (**Kerr** et al, unpublished).

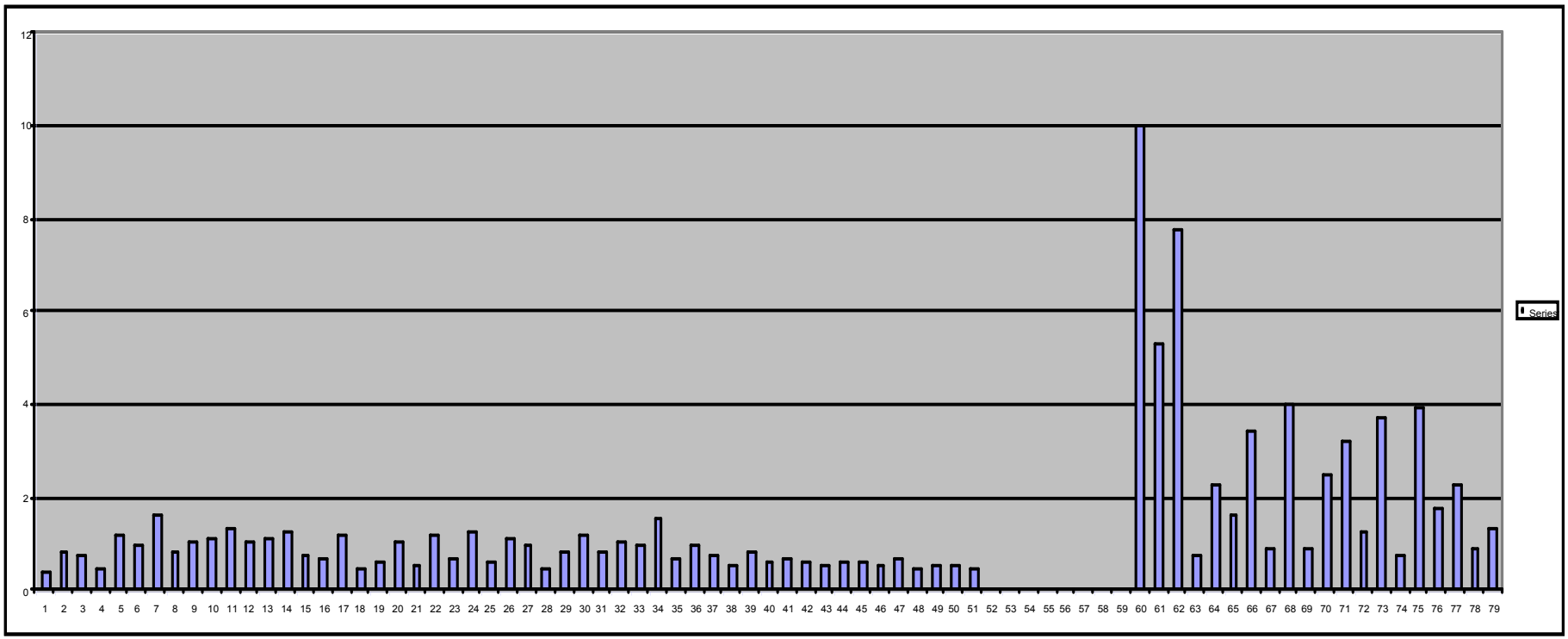
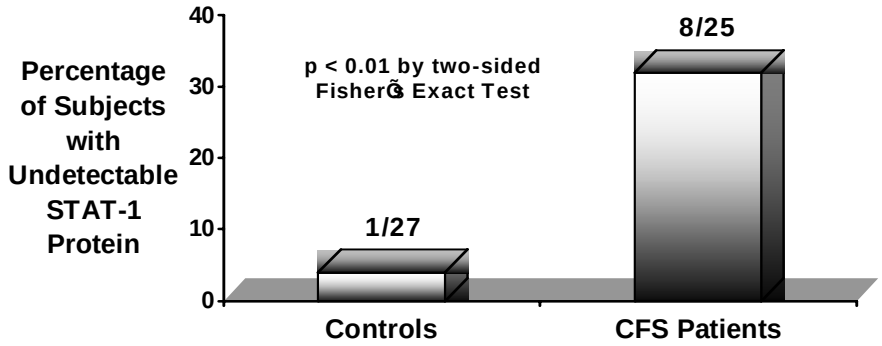


Table 4. Summary of preliminary study of CFS patients with cTCRG rearrangement and MCL.

#	ID#	Age	Sex	HHV-6 PCR	HHV-6 Viral Load	TCR-γ Clonality	Lymphoma/ Cancer
1	3005	10	M	+	ND	+	-
2	3001	55	F	-	ND	-	-
3	1002	54	M	+	ND	-	-
4	3004	16	M	-	ND	-	-
5	3003	24	M	+	ND	-	-
6	1379	25	F	+	2000000	+	-
7	1904	41	M	+	ND	-	-
8	987	53	M	+	8658	+	-
9	1566	53	F	+	105780	-	-
10	1282	58	M	+	187	+	MCL
11	1023	67	F	-	ND	-	-
12	1736	64	M	+	22	-	-
13	1078	60	M	+	928000	-	-
14	1987	85	M	-	ND	+	MCL
15	1726	64	F	+	63000	+	-
16	1014	58	M	+	6660	-	-
17	1761	49	M	-	ND	-	-
18	1631	45	F	+	7160	-	-
19	1094	54	F	+	4232	-	-
23	1281	68	M	+	29210	-	-
24	1614	69	F	+	ND	+	-
26	1815	50	F	-	ND	+	-
27	1689	48	M	+	39795	-	-
28	1380	77	M	+	6827	-	-
29	1164	55	F	+	932	-	-
30	1143	66	F	+	50770	+	-
36	1857	51	M	+	9807	-	-
38	1777	49	M	+	1309100	-	-
42	1127	62	F	+	ND	+	MCL
43	2591	42	F	+	ND	-	Parotid tumor
44	2152	75	M	-	ND	+	MCL
45	1674	66	F	+	ND	+	MCL
46	1849	56	M	+	ND	+	Hemangioma
47	1369	39	M	+	6277	-	-
48	1581	60	F	+	5162	-	-

ND= not detrmined. 35 of 50 patients tested are shown including all cTCRG= and MCL+

Figure 1. Recently Knox and Carrigan (unpublished) did a pilot study looking at STAT1 proteins in 25 CFS patients and 27 controls. Their results are summarized in the Figure below:



- TACK SA MYCKET!