

Presentation 9 – James Baraniuk

**“A Chronic Fatigue Syndrome Related
 Proteome in Cerebrospinal Fluid”**

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BMC Neurology 5:22, 2005

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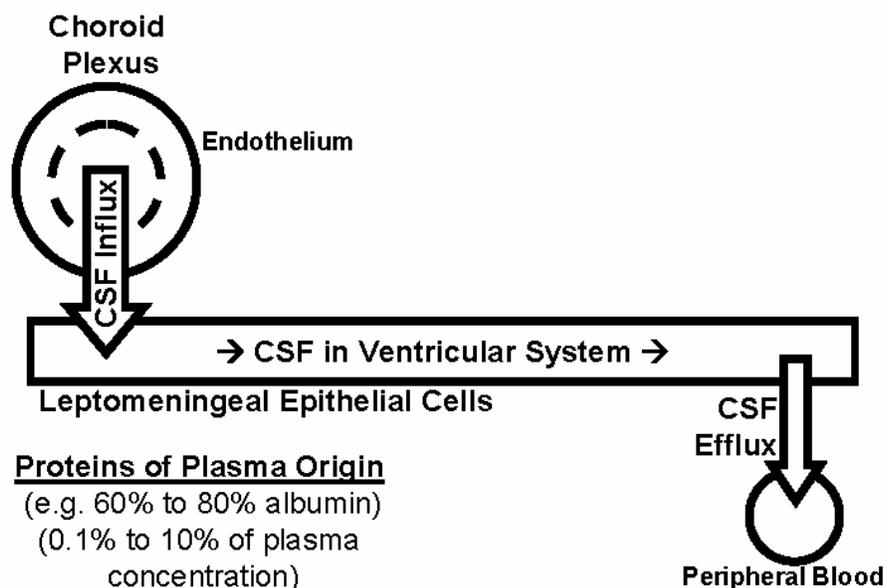
DNA → mRNA → Protein

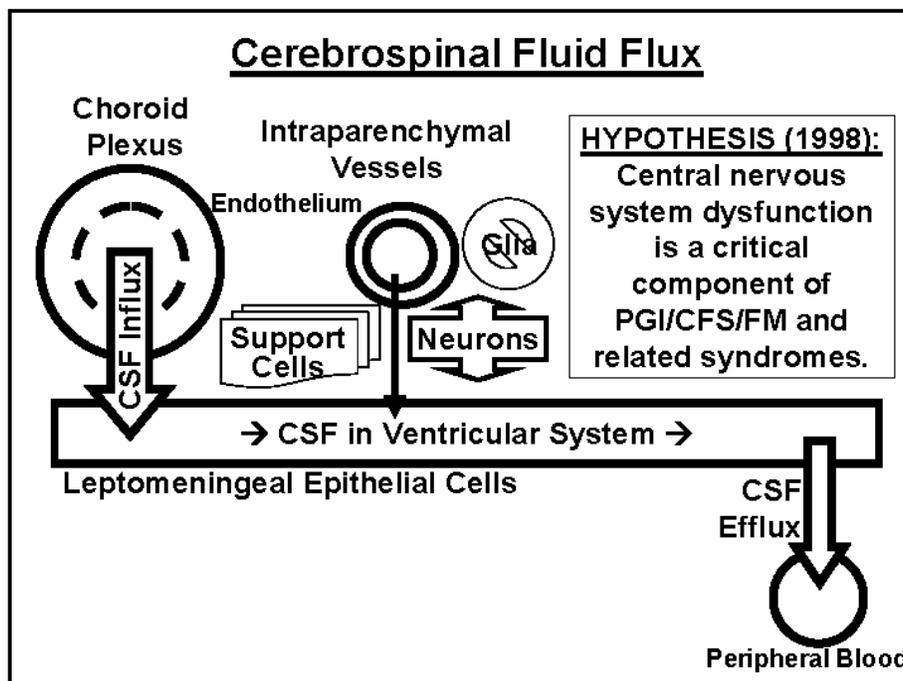
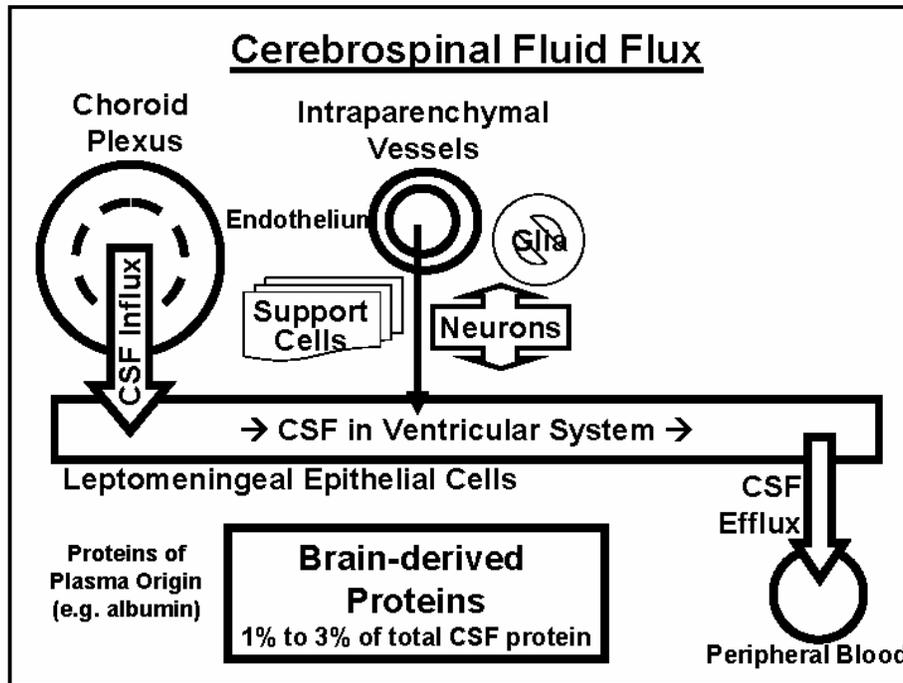
<u>Genomics</u>	<u>mRNA Microarray</u>	<u>Proteomics</u>
Examine genes in DNA Single point mutations (SNPs)	Examine mRNA expressed at one point in time	Examine the proteins in a cell, tissue, fluid sample
What you are born with	mRNA is made into proteins	Proteins determine what is happening now
Potential Risk Factors	Different expression between “Disease” and “Control”	Comparison of “Disease” and “Control”
Diathesis		Disease-related set of proteins or “Proteome”
Population Studies	Gene microarrays	

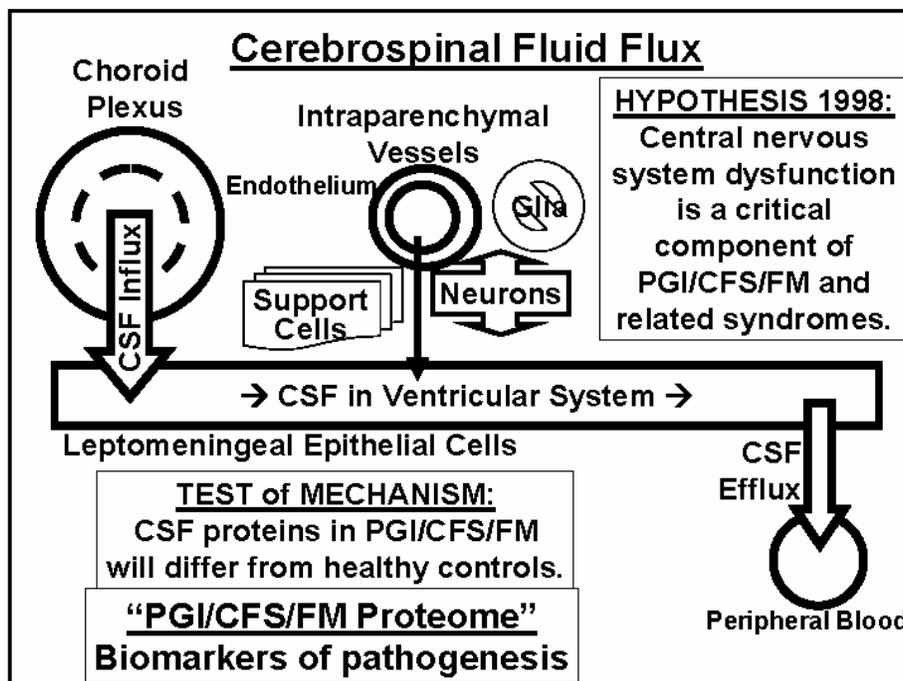
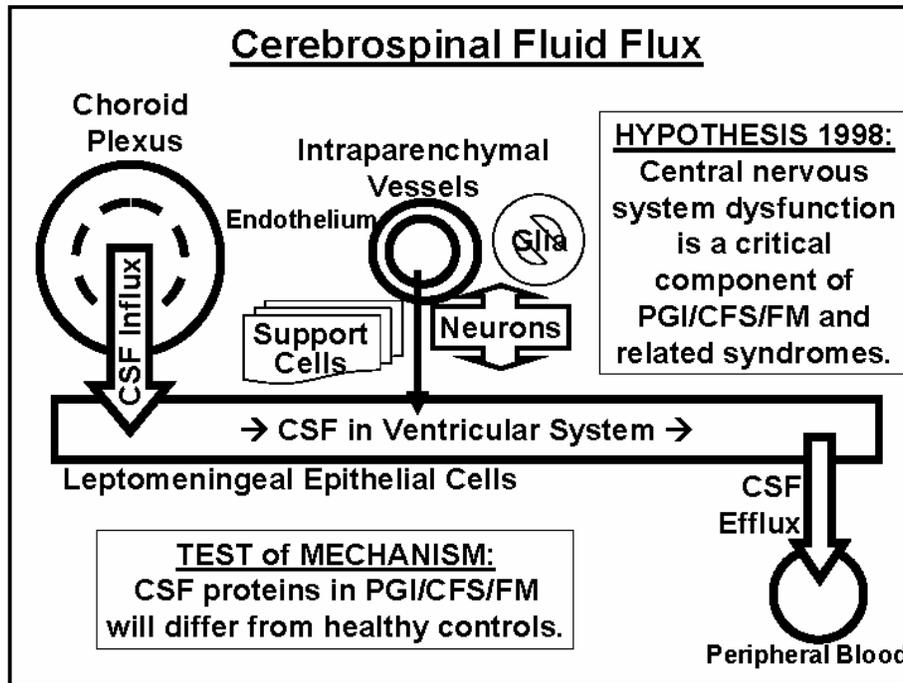
Outline

- Cerebrospinal fluid (CSF)
- Study design
- Patient groups for proteomic analysis
- Tandem Mass Spectrometry (MS-MS), Bioinformatics
- Statistical Analysis
- Implications
- Funding Sources
 - United States Department of Defense Award DAMD 170020018
 - Public Health Service Award RO1 AI42403
 - General Clinical Research Center Program 1 M01-RR13297-01A1
- Site:
 - Georgetown University G-CRC and Proteomics Laboratory

Where Does Cerebrospinal Fluid Come From?







Georgetown “CMI” Study; Dan Clauw, PI

- **Recruited Subject Groups:**
 - **Veterans** with Persian Gulf Illness (PGI, GWI, CMI)
 - **Fibromyalgia** (FM; positive controls, ACR Criteria)
 - **Healthy controls** (HC)

- **Multidimensional Evaluation:**
 - Psychiatric, psychometric
 - HPA axis, hyperalgesia, fMRI
 - Autonomic and exercise responses
 - Blood biomarker and lumbar puncture
 - Assess for PGI, CFS, FM, MCS, IBS, and other syndromes

Cerebrospinal Fluid

- One anesthetist for reproducible technique
- Lumbar punctures at same time of the morning
- Narrow gauge (22G) catheters
- Few, mild adverse events (headaches)

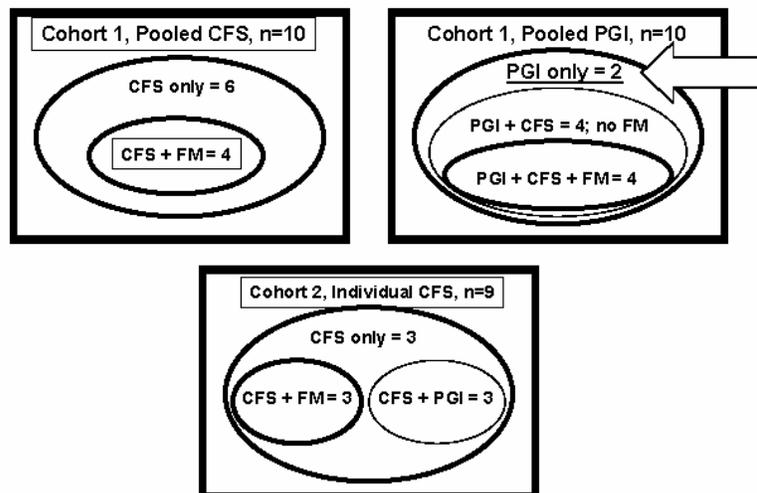
- Tubes 2, 3, 4
- Centrifuged to remove cells
- Aprotinin (antiprotease) added
- Frozen at -80°C

2 Distinct Proteomic Analysis Groups

- | | |
|---|--|
| <ul style="list-style-type: none">• <u>Cohort 1:</u>• <u>Pooled Samples</u>
• Healthy controls (HC)• PGI• CFS
• N = 10 CSF specimens per group• 3 samples | <ul style="list-style-type: none">• <u>Cohort 2:</u>• <u>Individual Samples</u>
• N= 12 HC• N = 9 "CFS"
• 21 separate proteomic analyses• Statistical comparisons |
|---|--|

Final overall analysis of all HC vs. all CFS/PGI/FM ("CFS")

Overlapping Syndromes: "Psycho – Semantics" of Case Definitions



Cohort Characteristics					
Group	N	Age (yr)	Male	CESD Affective Dysfunction	Pain Threshold (kg)
COHORT 1 (Pooled Samples)					
HC Pool	10	34.4 (29.1 to 39.7)	80%	4.3 (0.6 to 7.9)	7.69 (5.72 to 9.65)
CFS Pool	10	39.9 (34.3 to 45.5)	20% ^{***}	17.6 ^{***} (12.1 to 23.0)	4.01 ^{**} (2.86 to 5.16)
PGI Pool	10	43.5 (38.7 to 48.3)	60%	18.1 ^{**} (8.7 to 27.5)	4.89 [*] (3.64 to 6.14)
COHORT 2 (Individual Samples)					
HC	12	41.3 (33.6 to 48.9)	75%	-	7.17 (5.71 to 8.64)
CFS	9	39.1 (32.2 to 46.0)	33%	-	4.97 [§] (3.75 to 6.19)

^{*}p<0.05, ^{**} p<0.01, ^{***} p<0.001 compared to HC Pool results; [§] p<0.05 compared to HC individuals; ANOVA followed by Student's t-tests.

Clinical Summary

- CFS / PGI / FM groups had extensive overlap, with only 2 “pure” PGI subjects.
- CFS was the single most common “syndrome” in these subjects.
- CFS / PGI / FM subjects had:
 - Worse QOL (SF-36), fatigue (MFI), and affective dysfunction (CESD)
 - Lower pain thresholds (systemic hyperalgesia)

Proteomics: Proteins → Peptides

- CSF proteins digested into peptides with trypsin
- Trypsin peptides separated by capillary liquid chromatography (CapLC)
- → Tandem mass spectrometry (MS-MS)
 - 1st MS: quadrupole MS to separate peptide ions
 - 2nd MS-MS: time-of-flight MS to sequence peptides

Peptide Sequences to Protein Functions

- 2nd MS-MS spectra → sequence each peptide
- Peptide sequences → MASCOT software
- MASCOT → protein identification for each sample

- Protein functions and interactions →
- Protein Information Resource (PIR)
- <http://pir.georgetown.edu>

Proteins from Pooled Samples
(Cohort 1)

Proteins that were detected in **BOTH** the
pooled PGI and pooled CFS specimens

AND

were **ABSENT** from the pooled healthy
control specimen

defined the

“Cohort 1 CFS-related Proteome”

Cohort 1 Pooled CFS” Proteome

Cohort 1
“Pooled CFS” Proteome

α 2-Macroglobulin
Ceruloplasmin / ferroxidase II
Orosomuroid 2
Autotaxin / phosphodiesterase 1 α
Amyloid precursor-like protein 1
BEHAB

Complement C4A, C4B
PEDF
Gelsolin
Carnosine dipeptidase 1 (CNDP1)

Proteins from Individual Samples
(Cohort 2)

- **Statistical analysis**
- Lists of proteins from each individual sample
 - **Multilogistic analysis and modeling (GLM)**
 - **Support Vector Machine Learning (SVM-PSO-LOO)**
- Identify the unique set of proteins found in CFS/PGI/FM but not healthy controls

Proteins from Individual Samples
(Cohort 2)

- **“Detectability”:**
 - All proteins detected and identified by 2nd MS-MS.
 - Peptides identified above the lower limits of detection
- **Frequency of detection:**
 - The frequencies or prevalences of each protein in the healthy control group (HC) and CFS/PGI/FM group.
 - Qualitative analysis (ANOVA).
- Proteins detected significantly more frequently in CFS/PGI/FM than HC group formed the:
“CFS/PGI/FM related proteome”.

Cohort 2 “CFS/PGI/FM” Proteome

**Cohort 2
 “CFS” Proteome**

Keratin 16
 α2-Macroglobulin
 Ceruloplasmin / ferroxidase II
 Orosomuroid 2
 Autotaxin / phosphodiesterase 1α
 Amyloid precursor-like protein 1
 BEHAB
 Keratin 6C
 Keratin 17
 Orosomuroid 1
 Keratin 10
 Complement C4B
 PEDF
 Gelsolin
 Carnosine dipeptidase 1 (CNDP1)
 Keratin 14

Comparison of Cohort 1 and 2 Proteomes

Cohort 1 “Pooled CFS” Proteome	Cohort 2 “CFS” Proteome
	Keratin 16
α2-Macroglobulin Ceruloplasmin / ferroxidase II Orosomuroid 2 Autotaxin / phosphodiesterase 1α Amyloid precursor-like protein 1 BEHAB	α2-Macroglobulin Ceruloplasmin / ferroxidase II Orosomuroid 2 Autotaxin / phosphodiesterase 1α Amyloid precursor-like protein 1 BEHAB
	Keratin 6C Keratin 17 Orosomuroid 1 Keratin 10
Complement C4A, C4B PEDF Gelsolin Carnosine dipeptidase 1 (CNDP1)	Complement C4B PEDF Gelsolin Carnosine dipeptidase 1 (CNDP1)
	Keratin 14

Odds of matching 10 proteins: 10^{-15}

Multilogistic Proteomic Biosignature (B1/5) Model

IF any 1 of these 5 proteins was detected:

Keratin 16
 α 2-Macroglobulin
Orosomuroid 2
Autotaxin / phosphodiesterase 1 α
Pigment Epithelium Derived Factor (PEDF)

THEN

CFS was present with
OR=34.5
(1.49 to 809.61; $p=0.0072$, Fisher's Exact test)

AND

CFS status = gender + (B1/5)
80% concordance

First objectively defined, predictive model
for these illnesses.

Pathophysiological Implications

Protease – Antiprotease
Imbalance

- α 2-Macroglobulin
- Orosomuroid 1 and 2

Structural Injury

- Gelsolin (apoptosis)
- Amyloid APLP1
- C4B (C3)

Oxidant Injury

- Ceruloplasmin
- Carnosine dipeptidase 1

Vascular Dysregulation

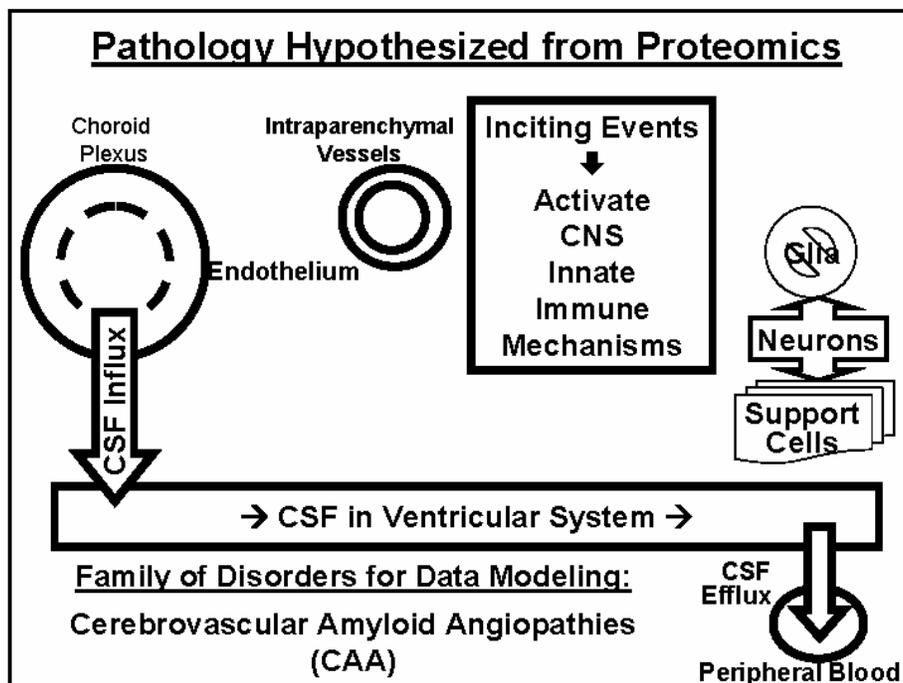
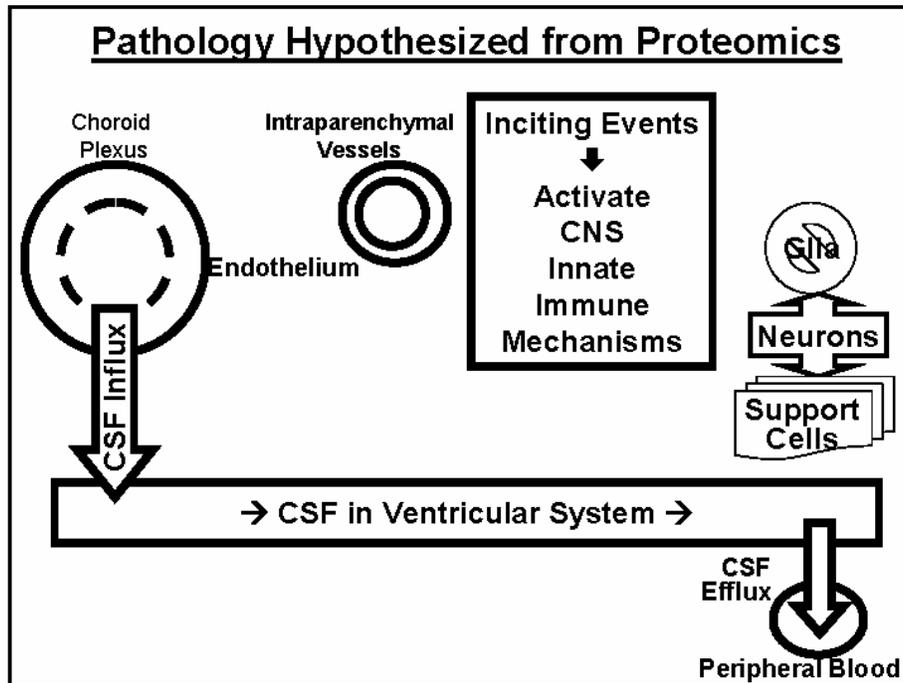
- Autotaxin
- Pigment Epithelium Derived Factor (EPDF)
- Vasoconstriction (ischemia)
- Endothelial proliferation (repair)

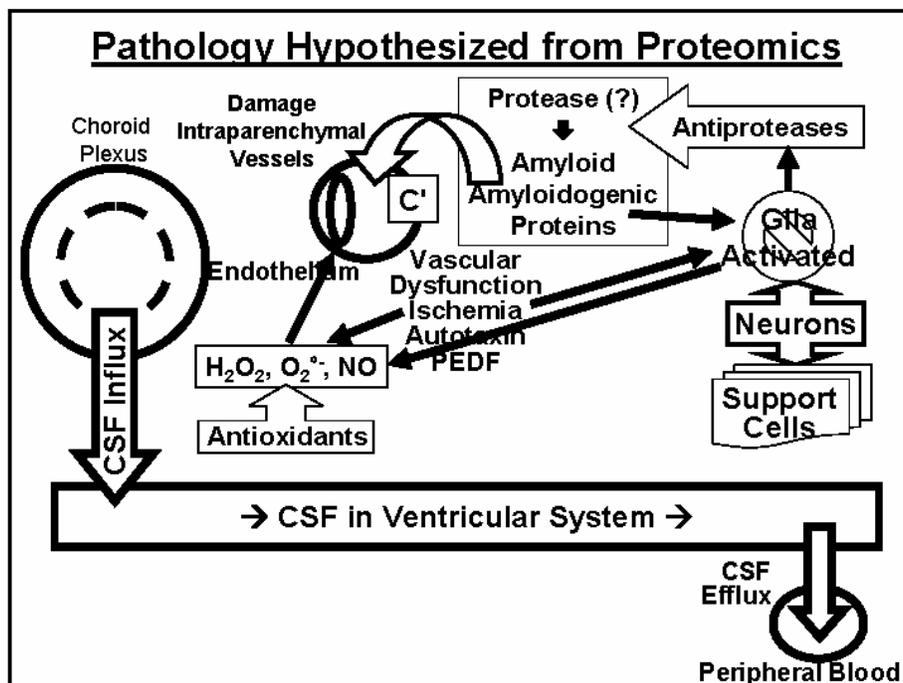
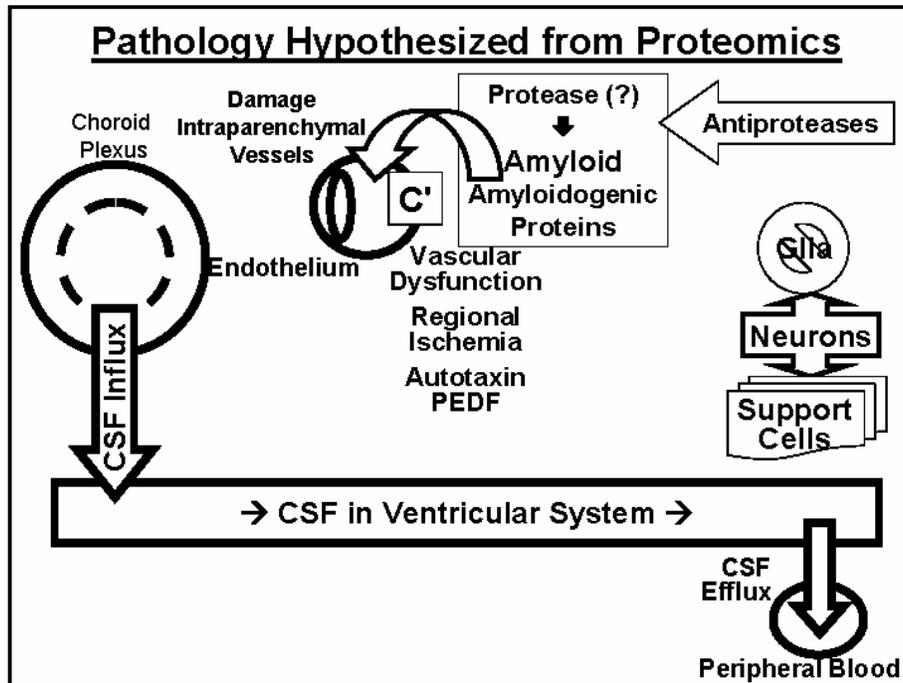
Leptomeningeal Activation

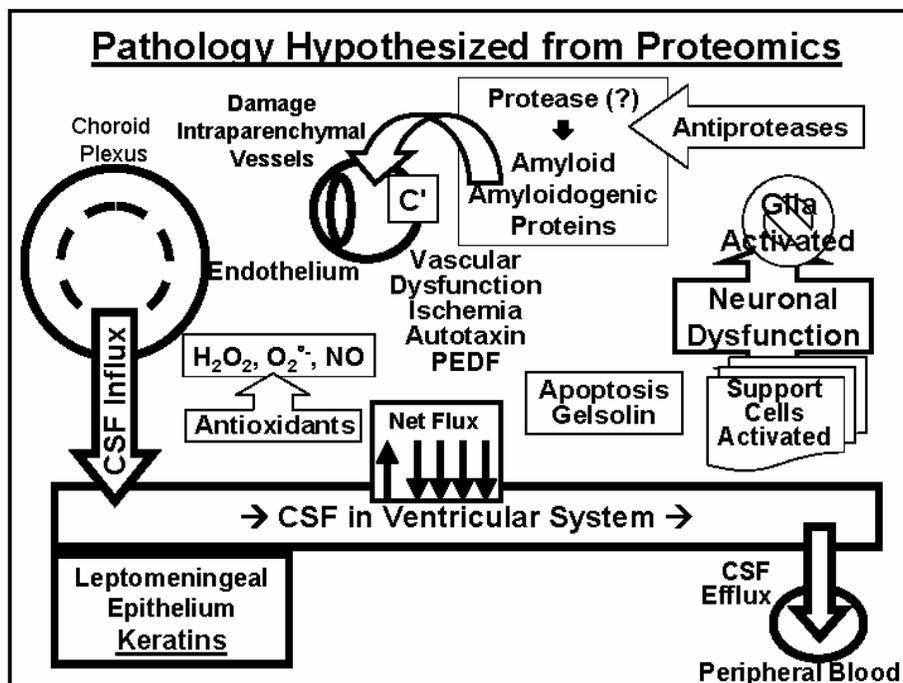
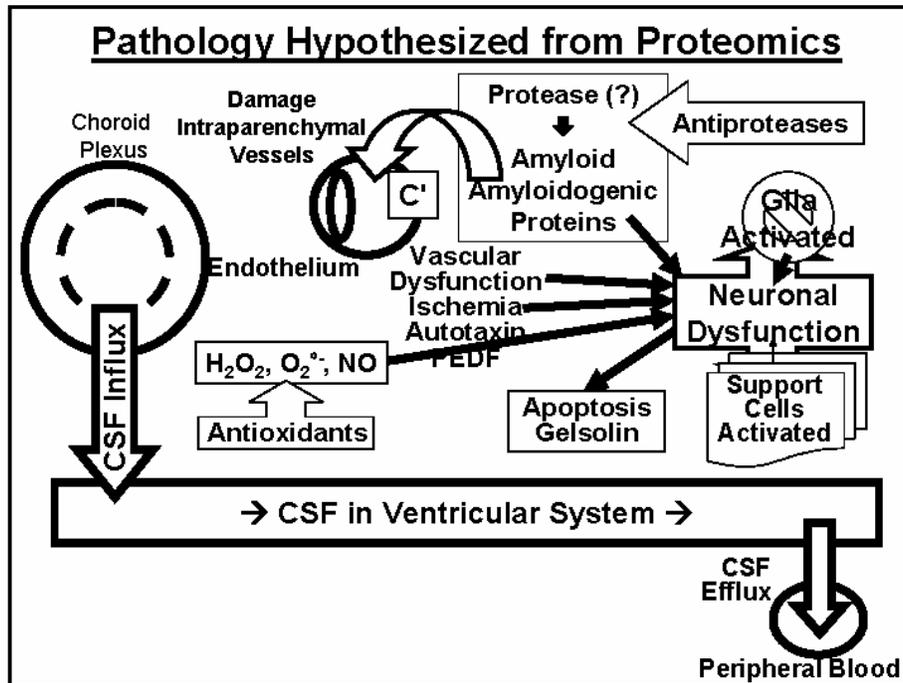
- Keratins 4, 10, 16, 17

Structural Repair

- Brain-enhanced hyaluronan binding (BEHAB)



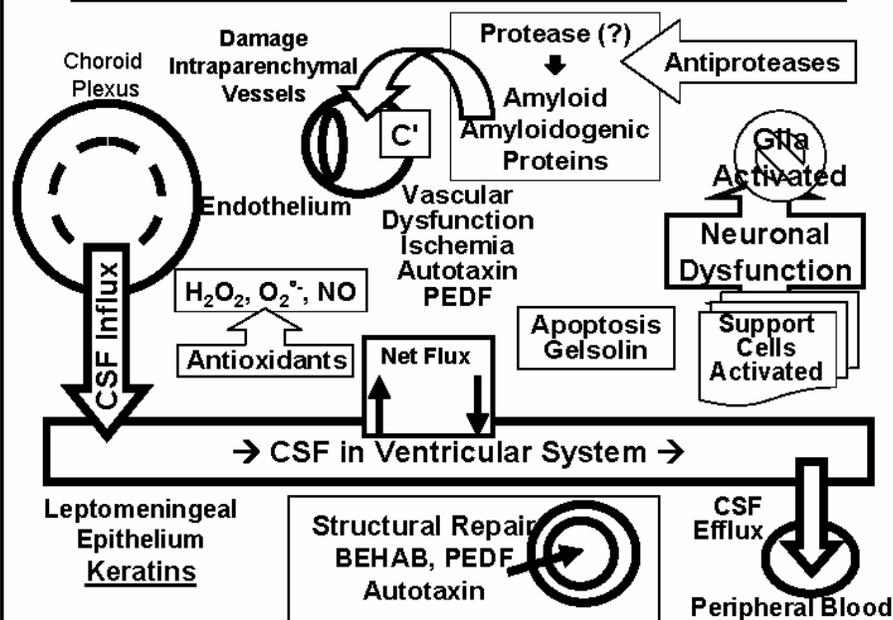




Conclusions: Proteomic Modeling

- Proteomic analysis of 2 different cohorts of CFS/PGI/FM subjects qualitatively identified a subset of cerebrospinal fluid proteins.
 - “CFS/PGI/FM Proteome”
- Multilogistic modeling identified a biosignature (**B1/5**) where the presence of 1 out of 5 proteins was sufficient to predict CFS status.
- This is the first objectively defined model predicting CFS/PGI/FM status.
 - OR=34.5; 80% concordance

Conclusions: Reversible, Non-Lethal CAA?



<u>DNA → mRNA → Protein</u>		
<u>Genomics</u>	<u>mRNA Microarray</u>	<u>Proteomics</u>
<ul style="list-style-type: none"> •Examine genes in DNA •Single point mutations (SNPs) •What you are born with •Potential •Risk Factors •Diathesis •<u>Population Studies</u> 	<ul style="list-style-type: none"> •Examine mRNA expressed at one point in time •mRNA is made into proteins •Different expression between "Disease" and "Control" •<u>Gene microarrays</u> 	<ul style="list-style-type: none"> •Examine the proteins in a cell, tissue, fluid sample •Proteins determine what is happening now •Comparison of "Disease" and "Control" •Disease-related •<u>"Proteome"</u>
<div style="border: 1px solid black; padding: 5px; width: fit-content; margin: 0 auto;"> <p><u>Snap shots of one point in time.</u> Poor agreement (17%) RNAi Post-translational modifications</p> </div>		