Presentation 9 – James Baraniuk

"A Chronic Fatigue Syndrome Related Proteome in Cerebrospinal Fluid"

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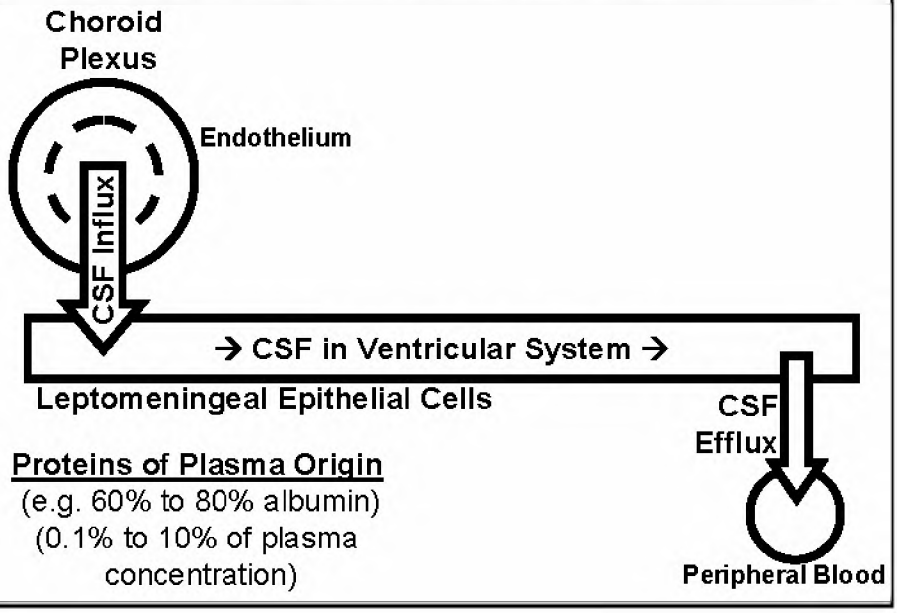
DNA → mRNA → Protein

Genomics	mRNA Microarray	Proteomics
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	

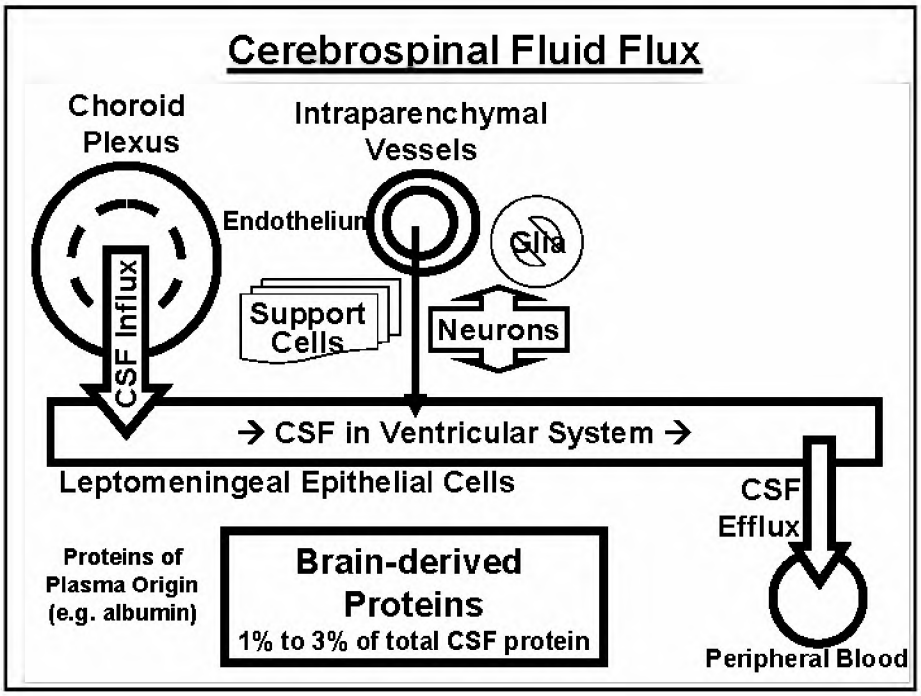
Outline

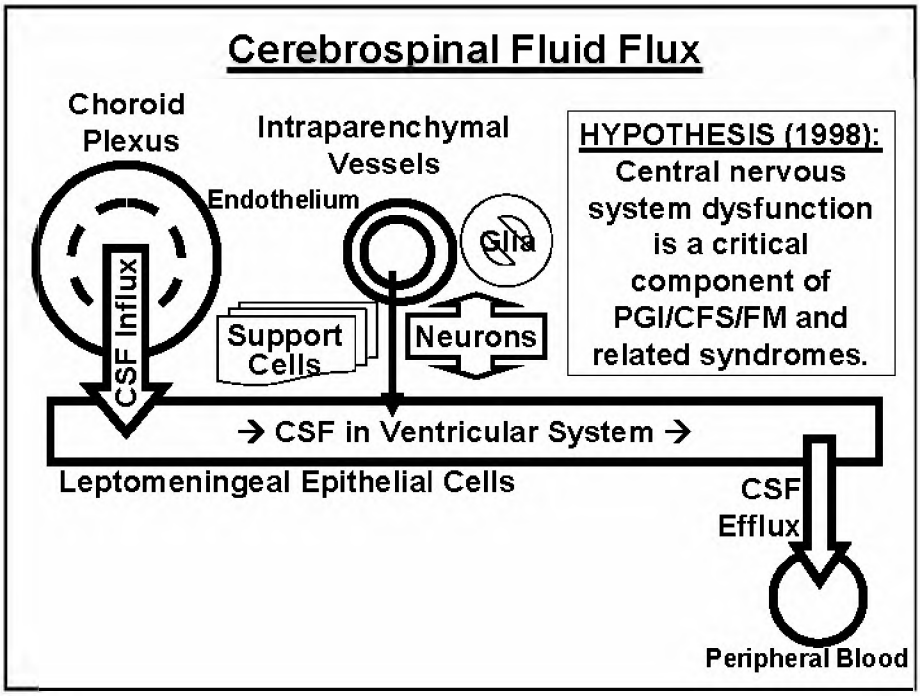
- Cerebrospinal fluid (CSF)
- Study design
- Patient groups for proteomic analysis
- Tandem Mass Spectrometry (MS-MS), Bioinformatics
- Statistical Analysis
- Implications
- Funding Sources
  - United States Department of Defense Award DAMD 170020018
  - Public Health Service Award RO1 AI42403
  - General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

Where Does Cerebrospinal Fluid Come From?



Proteins of Plasma Origin
(e.g. 60% to 80% albumin)
(0.1% to 10% of plasma concentration)


Cerebrospinal Fluid Flux
Choroid Plexus
Intraparenchymal Vessels
Endothelium
Glia
CSF Influx
Support Cells
Neurons
→ CSF in Ventricular System →
Leptomeningeal Epithelial Cells
CSF Efflux
Proteins of Plasma Origin (e.g. albumin)
Brain-derived Proteins
1% to 3% of total CSF protein
Peripheral Blood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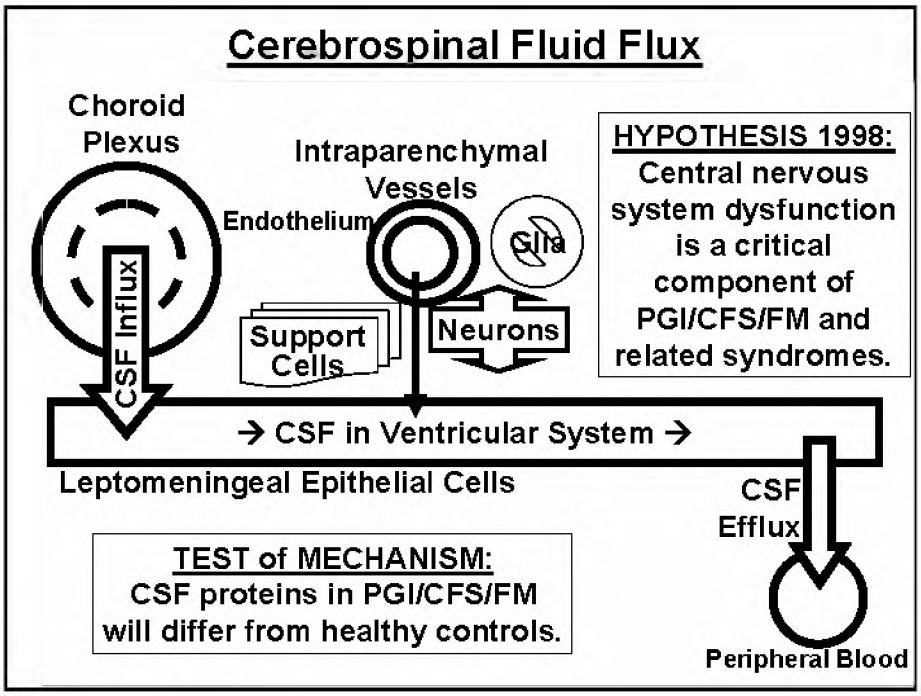
HYPOTHESIS (1998):
Central nervous system dysfunction is a critical component of PGI/CFS/FM and related syndromes.
→ CSF in Ventricular System →
Leptomeningeal Epithelial Cells
CSF Efflux
Peripheral Blood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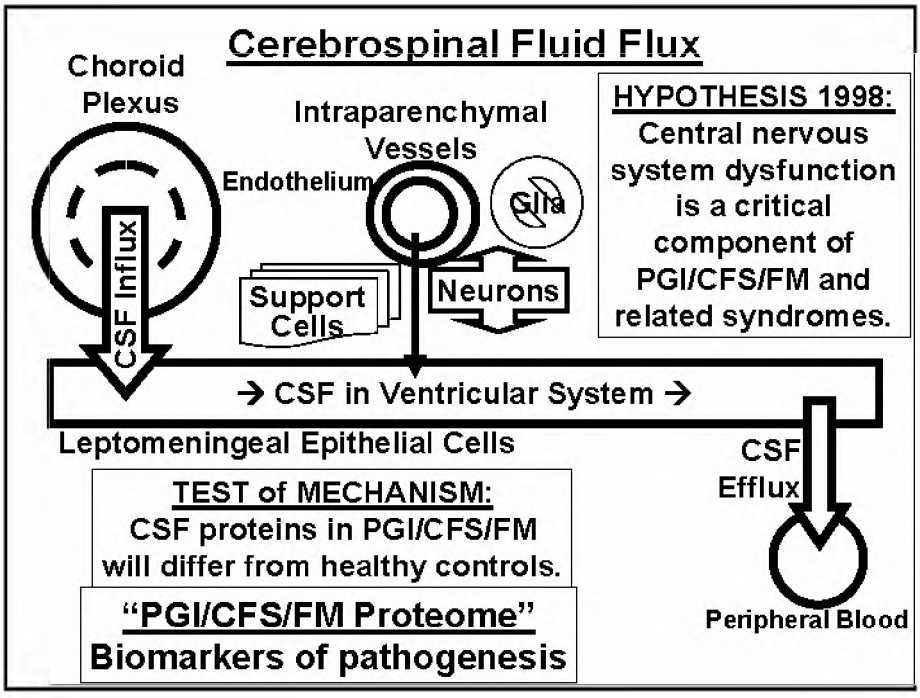
CSF proteins in PGI/CFS/FM will differ from healthy controls.
Peripheral Blood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TEST of MECHANISM:
CSF proteins in PGI/CFS/FM will differ from healthy controls.
"PGI/CFS/FM Proteome"
Biomarkers of pathogenesis
Peripheral Blood

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)

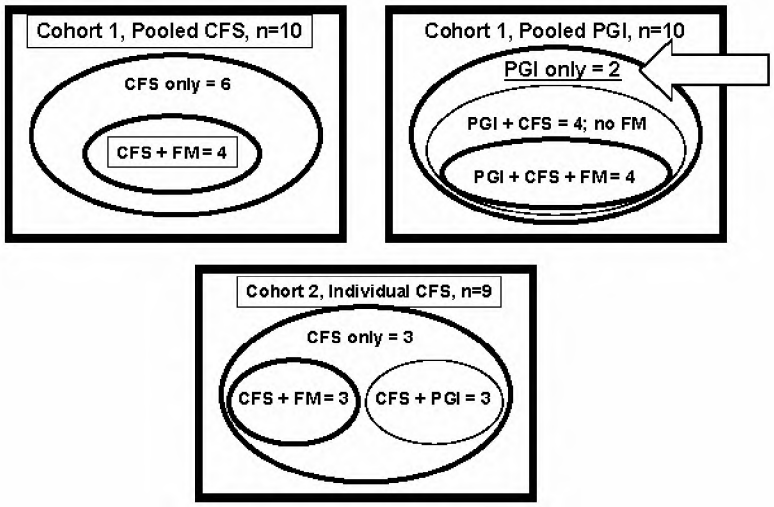
- Tubes 2, 3, 4
- Centrifuged to remove cells
- Aprotinin (antiprotease) added
- Frozen at -80°C

2 Distinct Proteomic Analysis Groups

Cohort 1: Pooled Samples	Cohort 2: Individual Samples
• Healthy controls (HC) • PGI • CFS	• N= 12 HC • N = 9 "CFS"
• N = 10 CSF specimens per group • 3 samples	• 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

α2-Macroglobulin
Ceruloplasmin / ferroxidase II
Orosomucoid 2
Autotaxin / phosphodiesterase 1α
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
Orosomucoid 2
Autotaxin / phosphodiesterase 1α
Amyloid precursor-like protein 1
BEHAB
Keratin 6C
Keratin 17
Orosomucoid 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	**α2-Macroglobulin**
Ceruloplasmin / ferroxidase II	**Ceruloplasmin / ferroxidase II**
Orosomucoid 2	**Orosomucoid 2**
Autotaxin / phosphodiesterase 1α	**Autotaxin / phosphodiesterase 1α**
Amyloid precursor-like protein 1	**Amyloid precursor-like protein 1**
BEHAB	**BEHAB**
	Keratin 6C
	Keratin 17
	Orosomucoid 1
	Keratin 10
Complement C4A, C4B	**Complement C4B**
PEDF	**PEDF**
Gelsolin	**Gelsolin**
Carnosine dipeptidase 1 (CNDP1)	**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
α2-Macroglobulin
Orosomucoid 2
Autotaxin / phosphodiesterase 1α
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
- α2-Macroglobulin
- Orosomucoid 1 and 2

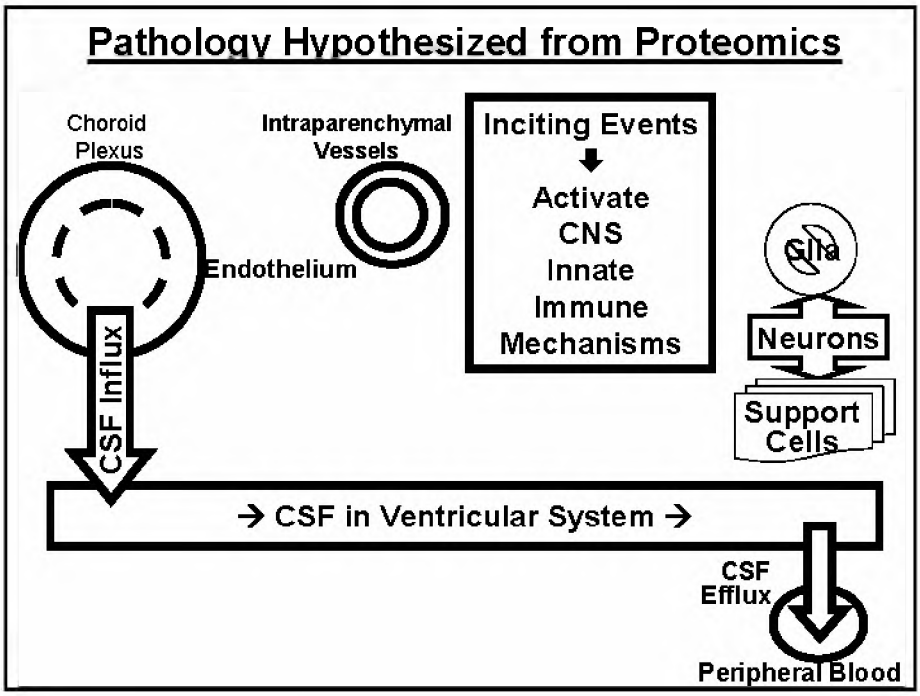
Structural Injury
- Gelsolin (apoptosis)
- Amyloid APLP1
- C4B (C3)

Oxidant Injury
- Ceruloplasmin
- Carnosine dipeptidaase 1

Vascular Dysregulation
- Autotaxin
- Pigment Epithelium Derived Factor (EPDF)
- Vasoconstrction (ischemia)
- Endothelial proliferation (repair)

Leptomeningeal Activation
- Keratins 4, 10, 16, 17

Structural Repair
- Brain-enhanced hyaluronan binding (BEHAB)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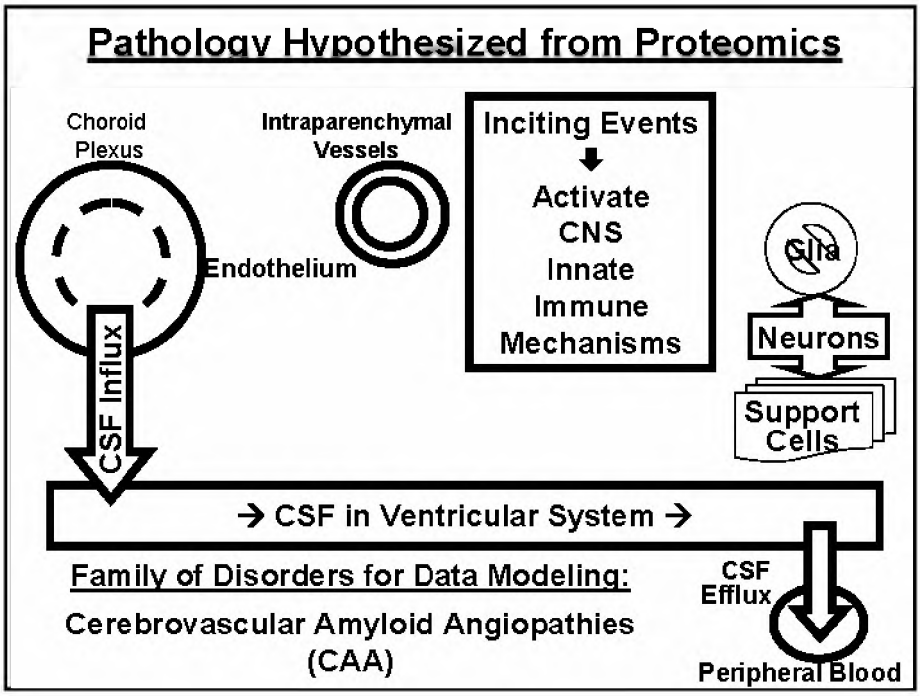
Choroid Plexus
Intraparenchymal Vessels
Endothelium
Inciting Events
Activate CNS Innate Immune Mechanisms
Glia
Neurons
Support Cells
CSF Influx
→ CSF in Ventricular System →
CSF Efflux
Peripheral Blood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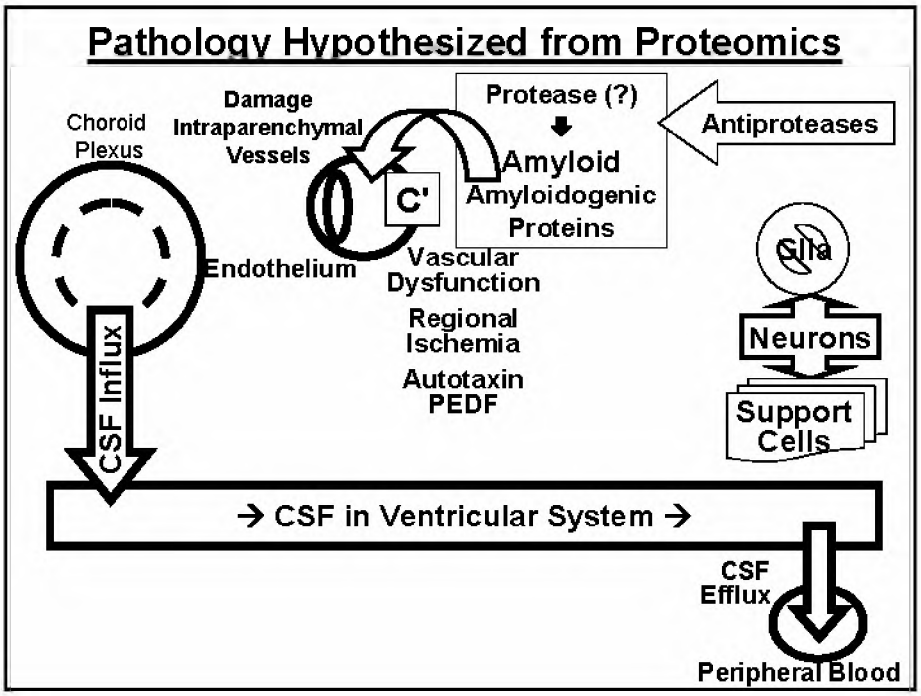
Family of Disorders for Data Modeling:
Cerebrovascular Amyloid Angiopathies (CAA)
CSF Efflux
Peripheral Blood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Protease (?)
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Amyloid
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C'
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Vascular Dysfunction
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Autotaxin
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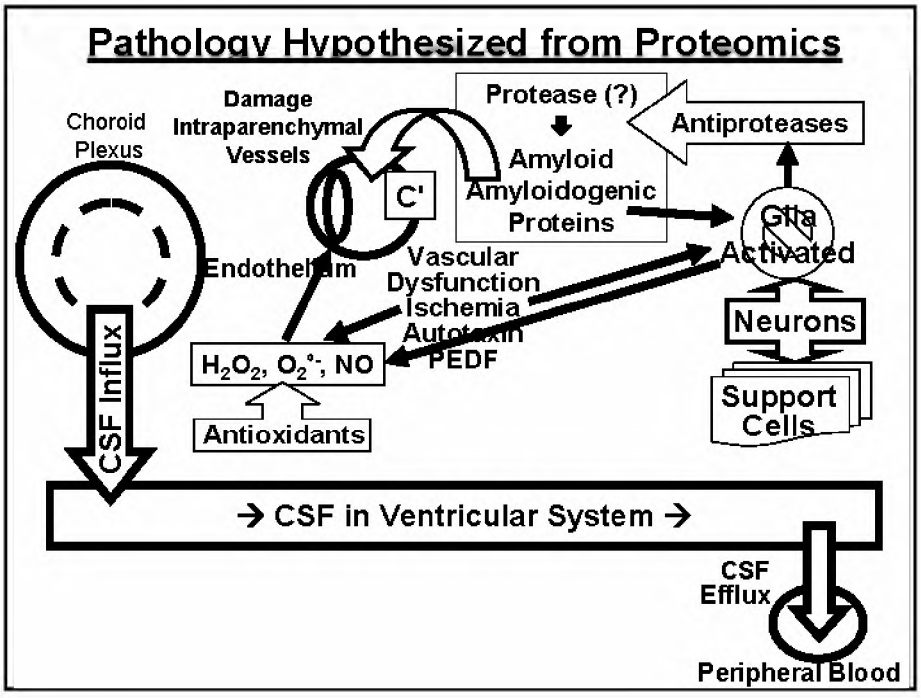
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Ischemia
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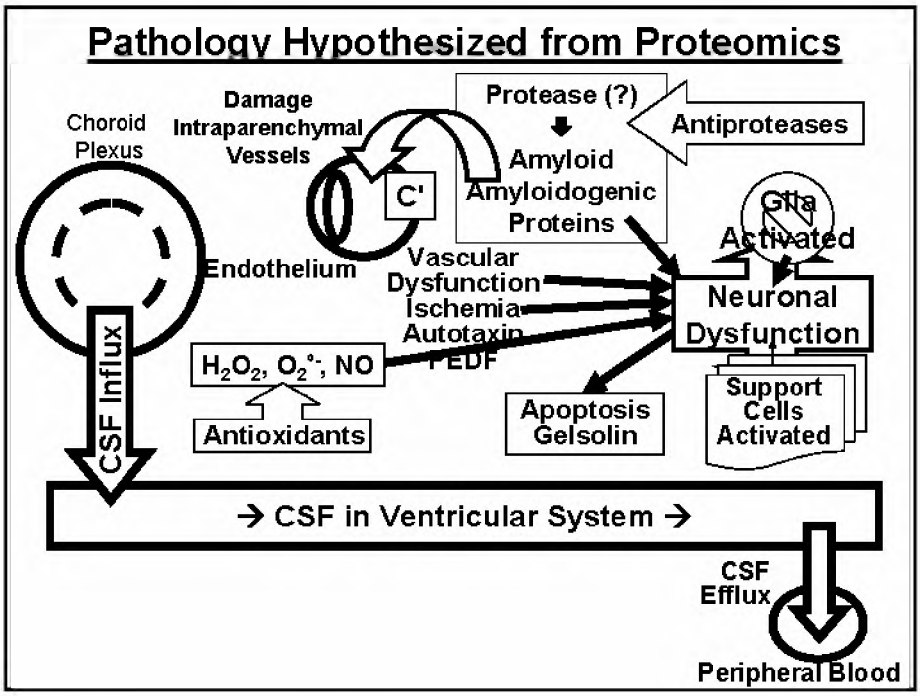
Activated
Neurons
Support Cells
H2O2, O2°-, NO
Antioxidants
CSF Influx
→ CSF in Ventricular System →
CSF Efflux
Peripheral Blood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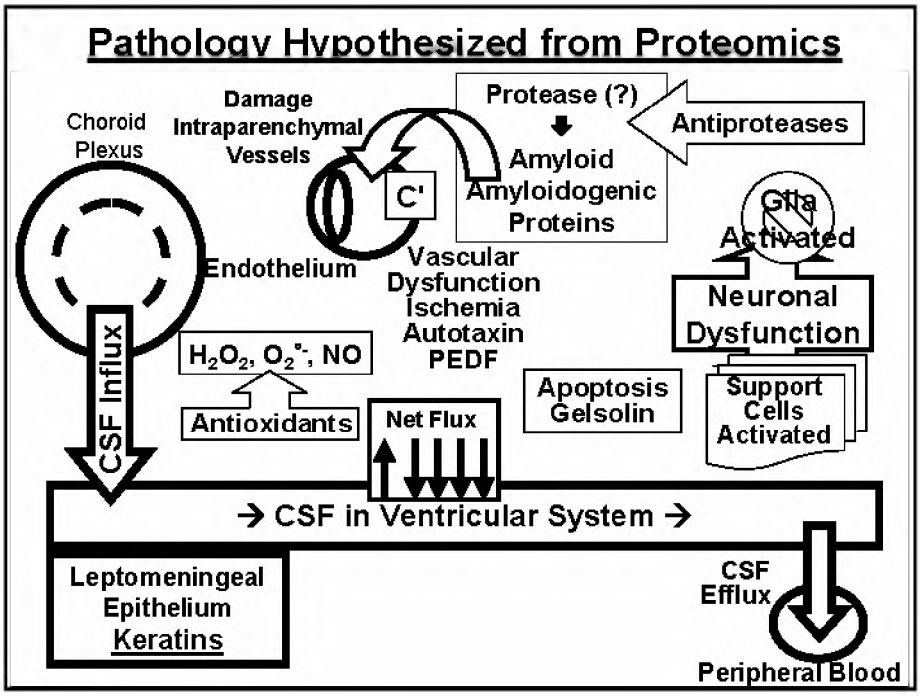
Neuronal Dysfunction
CSF Influx
H_2O_2, $O_2^{\bullet-}$, NO
Antioxidants
Apoptosis
Gelsolin
Support Cells Activated
→ CSF in Ventricular System →
CSF Efflux
Peripheral Blood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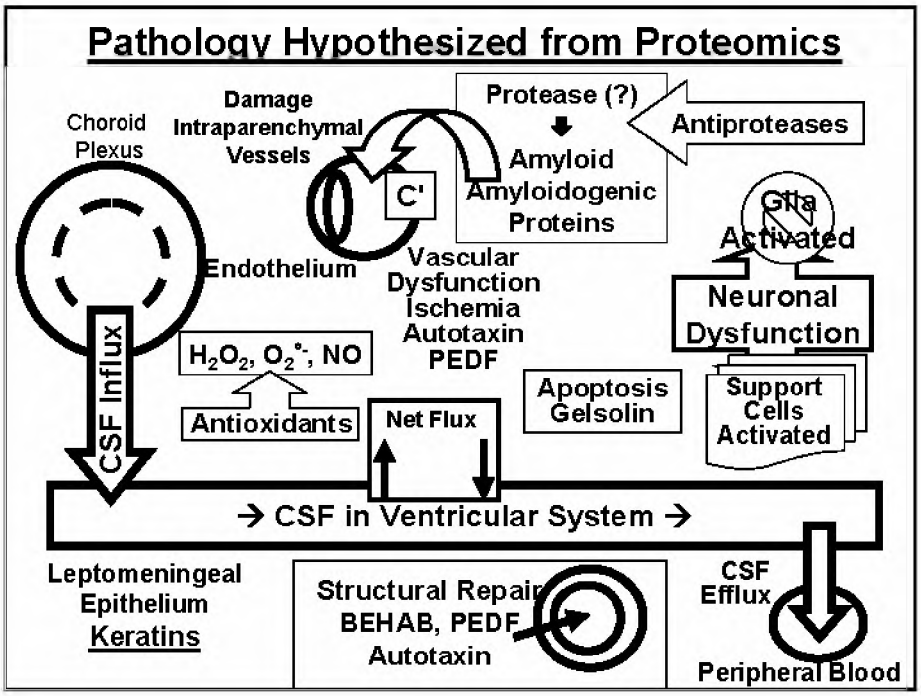


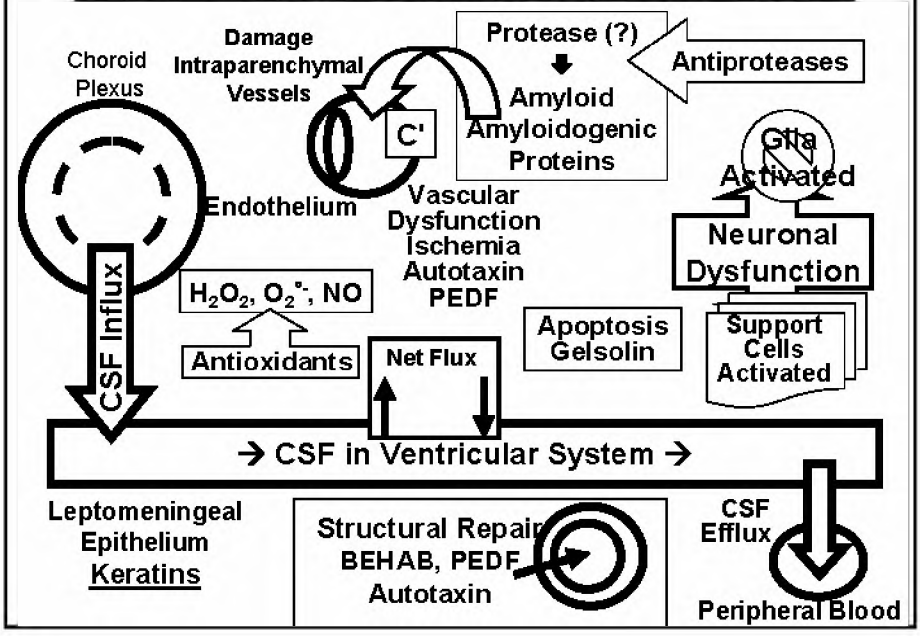
Conclusions: "Psycho-Semantics"

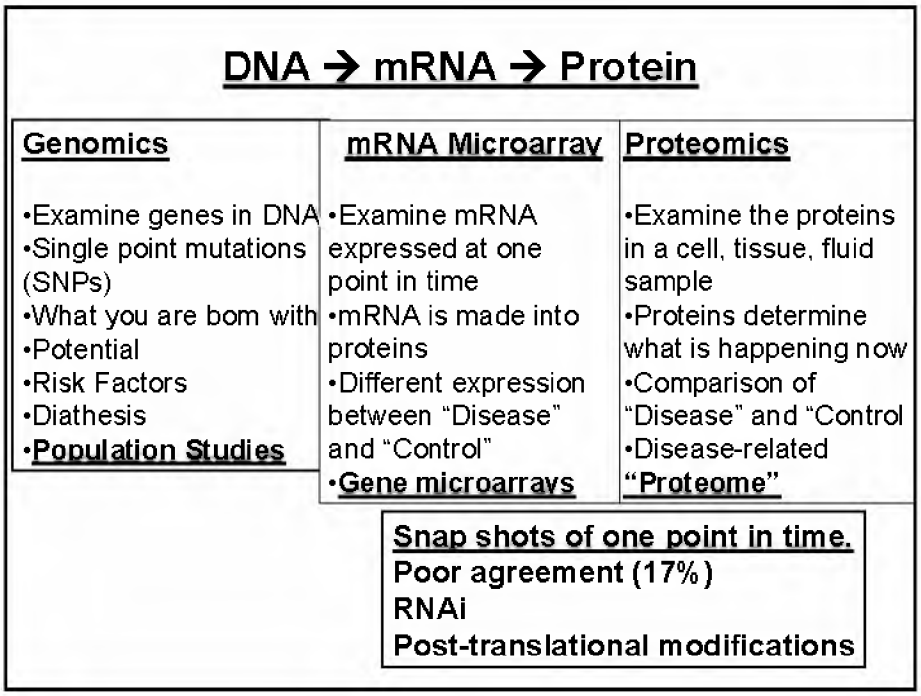
- Subjects met case designation criteria for several syndromes.
  - CFS / PGI / FM / IBS / MCS / hyperalgesia / dysautonomia . . .
- The high degree of overlap suggests that some pathophysiological mechanisms may be shared between syndromes.
- The patients are not "psycho" (the doctors are).

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?




DNA → mRNA → Protein
Genomics
•Examine genes in DNA
•Single point mutations (SNPs)
•What you are born with
•Potential
•Risk Factors
•Diathesis
•Population Studies
mRNA Microarray
•Examine mRNA expressed at one point in time
•mRNA is made into proteins
•Different expression between "Disease" and "Control"
•Gene microarrays
Proteomics
•Examine the proteins in a cell, tissue, fluid sample
•Proteins determine what is happening now
•Comparison of "Disease" and "Control
•Disease-related "Proteome"
Snap shots of one point in time.
Poor agreement (17%)
RNAi
Post-translational modifications