

Genetic and Genomic markers of CFS risk, progression and prognosis

CFSAC Day, Oct. 12th 2010

Alan R. Light, University of Utah,
Departments of Anesthesiology and
Neurobiology and Anatomy



Genetics and CFS

- Landmark-Høyvik, et al., The genetics and epigenetics of fatigue. PM R. 2010 May;2(5):456-65. PMID: 20656628
- “Although altered functioning in the hypothalamic-pituitary-adrenal axis,
- the serotonergic system,
- and associations with infectious agents have been identified,
- the search for genetic or epigenetic markers of fatigue, either in the context of CF or chronic fatigue syndrome (CFS) has been relatively unproductive or, in the case of epigenetics, nonexistent.”
- Maybe



- “To be able to confirm the hypothesis that risk for, or levels of, fatigue are influenced by the genetic or epigenetic background of an individual, studies need to be based on larger sample sizes with a more clearly defined phenotype.”
- I disagree with this statement. Genetics and genomics can actually help define the phenotype, as has been done with many other neurological diseases, including Alzheimer’s disease, Huntington’s Chorea, Spinocerebellar Ataxias, etc.
- “Studies need to focus not only on the influence of a single aspect such as single nucleotide polymorphisms (SNPs) or differential gene expression on disease risk or state, but also on the systems biology behind the disease in combination with information on environmental influences and validation of findings in functional studies.”
- I do agree with this last statement-with the caveat that one must know the systems in order to accomplish this



Limitation of existing Gene expression data not mentioned in many reviews

- All existing human data is from leukocytes, or worse, whole blood, (reticulocytes and platelets have RNA, and reticulocytes can contaminate columns).
- Leukocytes may not be the most important tissue affected by CFS.
- Symptoms are mostly associated with muscle and brain
- While muscle can be examined (one recent study does), it is difficult to obtain brain and sensory tissue from CFS patients (the majority of this will be postmortem, with the problem of RNA degradation).



Genetic associations with CFS

(from Landmark-Høyvik, et al., and others)

Immune system

- Smith et al (2005) HLA-DQA1*01 allele CFS
- Carlo-Stella et al (2006) increased *TNF-857* T allele and a decreased level of the *IFN* low producers
- Metzger et al (2008) protective effect of SNPs in IL17F

HPA-axis

- Smith et al (2006) subclasses of CFS associated with SNPs in *POMC*, *NR3C1*, *MAOA*, *MAOB*, and *TPH2*
- Rajeevan et al (2007) SNPs in *NR3C1* associated with risk for CFS
- Lee et al (2009) multiple SNPs in *NR3C1* for risk and gene expression differences may be causal in CFS and depression in CFS

Serotonergic system

- Narita et al (2003) longer allele variants in 5HTT (more transcription?) in CFS
- Smith et al (2008) 3 SNPs in *HTR2A* associated with CFS
- Ortega-Hernandez et al (2009) *HTR2A* associated with CFS and depression symptoms



Gene expression associations with CFS

- Gene expression alterations caused by environment can be much larger than gene expression changes caused by genetic mutations.
- Such alterations can occur very rapidly, and then recover or be very long lasting or permanent (epigenetics)
- mRNA increases (or decreases) do not necessarily reflect changes in proteins they code for. In only a very few cases have the gene products been related to the mRNA findings
- mRNA increases instead inform us of the transcriptional drive on the gene being assayed



Gene expression associations with CFS

Existing findings

- 25 publications from 6 independent labs since 2002, 14 of these since 2008
- 12 of these from data set from the CDC
(Using MGW 20K human array).



Genomic associations with CFS

No gene expression biomarkers for CFS found in **identical twin** study by Byrnes et al (2009)

Altered functioning in the **hypothalamic-pituitary-adrenal axis** and the **serotonergic** system were the predominant findings from the CDC data set.

88 genes differentially associated with CFS, and subgroup associations with specific viruses were found in CFS patients by the group headed by J.R. Kerr.



- Gene expression related to progression??
- Gene expression related to prognoses??
- **NONE**
- Gene expression related to severity

Presson et al., (2008) worked backwards using severity to help define genes related to CFS, but no prospective studies done



A different approach

- Almost all genetic and genomic studies done so far have focused on causes of Chronic Fatigue Syndrome
- The complaint about phenotype, and the “elephant and blind men” problems with CFS may be related to another problem:
- We don't really know what “Fatigue” is.



A different approach

- The genetic and genomic associations have already been helpful in understanding this phenomenon, but much more needs to be done.
- Fatigue means many different things to different people



A different approach

- For scientists, it has mostly meant the inability to contract skeletal muscle
- However, this is not the fatigue that CFS patients experience.
- Instead, they most commonly experience an overwhelming mental and physical tiredness that is a “sensory” experience.
- They “feel” fatigued, even though several groups have shown that they still have the ability to contract their muscles normally, at least during a single session.



A different approach

- Their “fatigue” and other symptoms can become dramatically increased following even a short duration of seemingly mild exercise.
- We postulate that this “sensory” fatigue comes in several flavors— two common ones,
 - 1) The perception of tiredness, heaviness, pressure, “lead leggedness” that accompanies prolonged muscle contraction—this is felt *before* the ache and burn we feel with sustained contraction
 - 2) The perception of lack of focus, confusion, ‘tiredness’ that accompanies exhausting mental tasks, other wise known as ‘mental fatigue’



A different approach

- As a result, we looked at mouse models to try to determine which molecular receptors on muscle sensory neurons might encode metabolites produced by contracting muscle associated with the sensation of muscle pain and fatigue
- Essentially, we are focusing “...on the systems biology behind the disease in combination with information on environmental influences” (in this case, exercise)



Light et al., 2008 Dorsal root ganglion neurons innervating skeletal muscle respond to physiological combinations of protons, ATP, and lactate mediated by ASIC, P2X and TRPV1. J Neurophysiol. 100: 1169-1170

- **ASIC** (ASIC3 or ASIC 2-3 heteromeres, or ASIC 1-2 heteromeres?) for both classes of sensory neurons, (both pain and fatigue)
- **P2X5** for high pH, low metabolite responses (fatigue) P2X2-3 heteromeres in humans?
- **P2X4** for low pH, high metabolite responses (pain)?
- **TRPV1** (the capsaicin receptor) for normal metabolite responses (heat detector, Linoleic acid metabolites?)



Adrenergic receptors

- β -2 and β -1 adrenergic receptors can enhance metabolite signaling from fatigue and pain receptors

Light KC, Bragdon EE, Grewen KM, Brownley KA, Girdler SS, Maixner W. (2009). Adrenergic dysregulation and pain with and without beta-blockade in women with fibromyalgia and temporomandibular disorder. *Journal of Pain* 10: 542-55.

- Cytokines can enhance metabolite signaling from these receptors
- adrenergic receptors and cytokines are upregulated by inflammation



Can we use this information for patients who have unexplained fatigue and/or muscle pain?

(funded by R21 from NINDS and by CFIDS and AFSA)

- Chronic Fatigue Syndrome-
- We determined that all of the critical genes are expressed on leukocytes
- Not really unexpected since leukocytes are also signaled by muscle contraction, and respond to muscle fatigue (home in on fatigued muscles).
- Leukocytes circulate through working muscles (and brain) and are exposed to metabolites



Brief Outline of Protocol

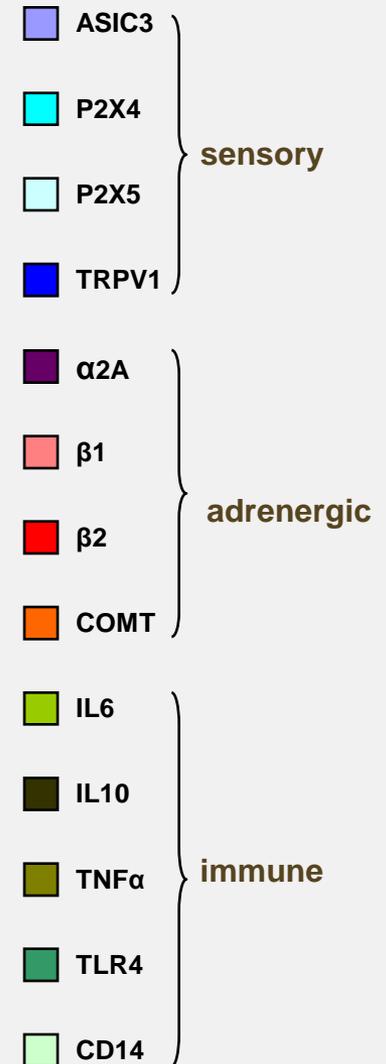
- 1. Screening to confirm that a physician (usually Dr. Bateman) has previously diagnosed CFS, current meds and if pain meds stopped for 3 days.
- 2. Testing: A) Baseline pre-exercise blood draw and Numerical Ratings of Mental and Physical Fatigue and Pain (0-100).
- B) Whole-body Airdyne bike exercise to 70% age predicted max HR for 25 min; HR and Work rate (kcal/kg/min) monitored every min, Ratings of Perceived Exertion (RPE, 0-10 VAS scale) every 5 min, BP monitored every 7 min, and Numerical Mental and Physical Fatigue and Pain ratings (0-100) at mid-task and end-task.
- C) Post-exercise blood draws at 0.5, 8, 24 and 48 hours with ratings of mental and physical fatigue and pain (0-100).
- D) Tender Point Exam and Questionnaires: ACR FMS Diagnostic Symptoms, CDC Symptom Inventory, MFI, MOS Short form-36, Beck Depression, State-trait Anxiety, Margoles Pain Chart



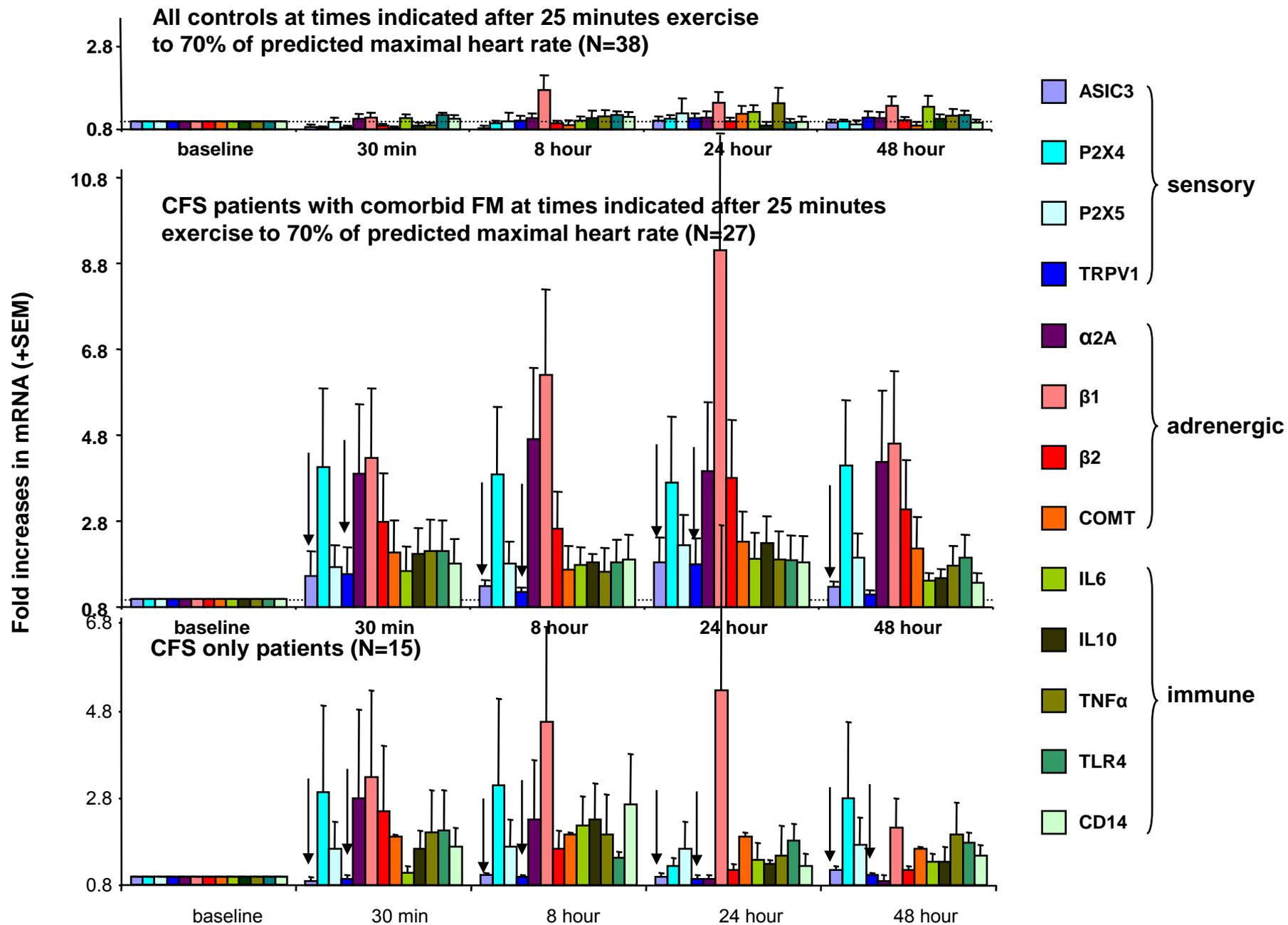
Gene Expression changes in CFS

- We looked at only 13 genes, that represent the sensory, adrenergic, and immune system
- We found no differential expression between controls and CFS patients of any of these genes at baseline

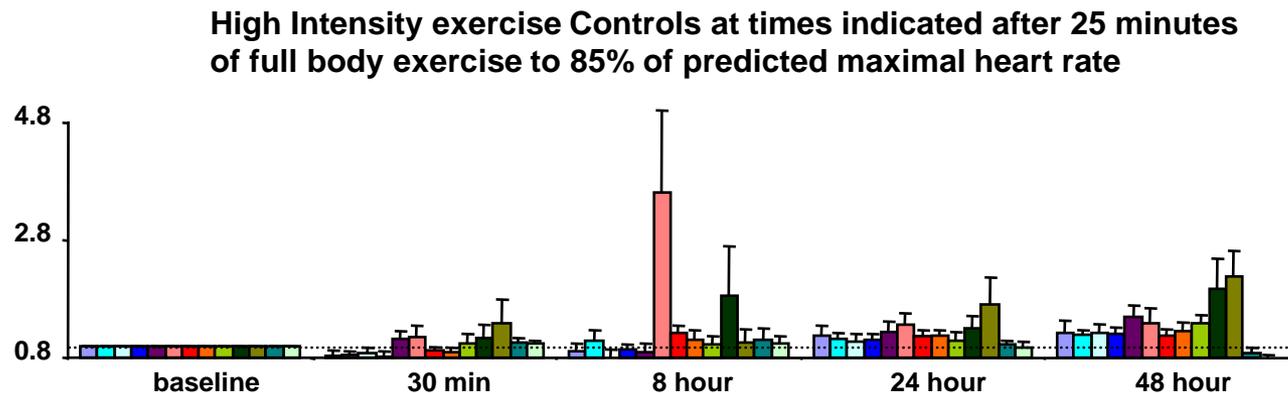
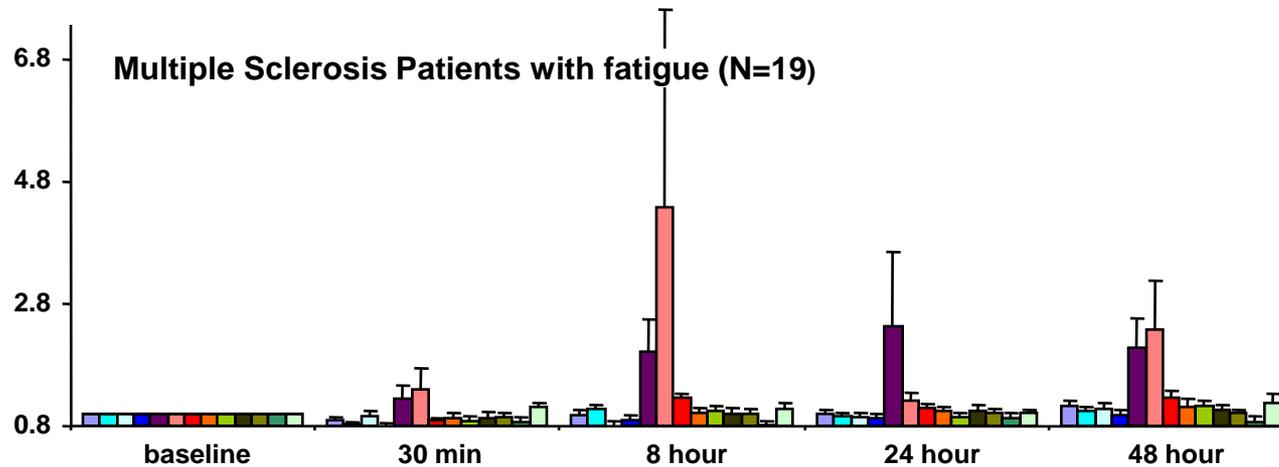
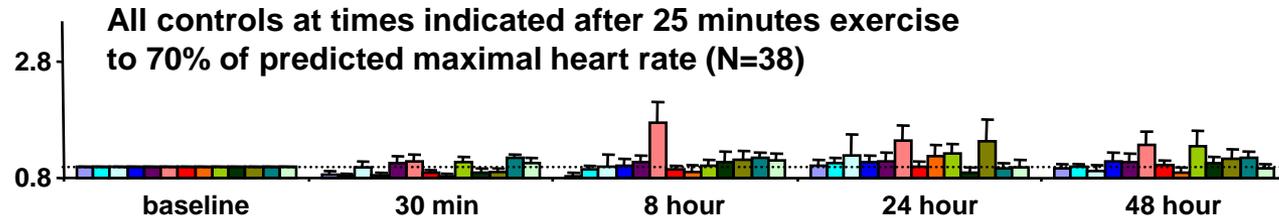
HOWEVER! Dramatic increases in gene expression seen in CFS and CFS+FM



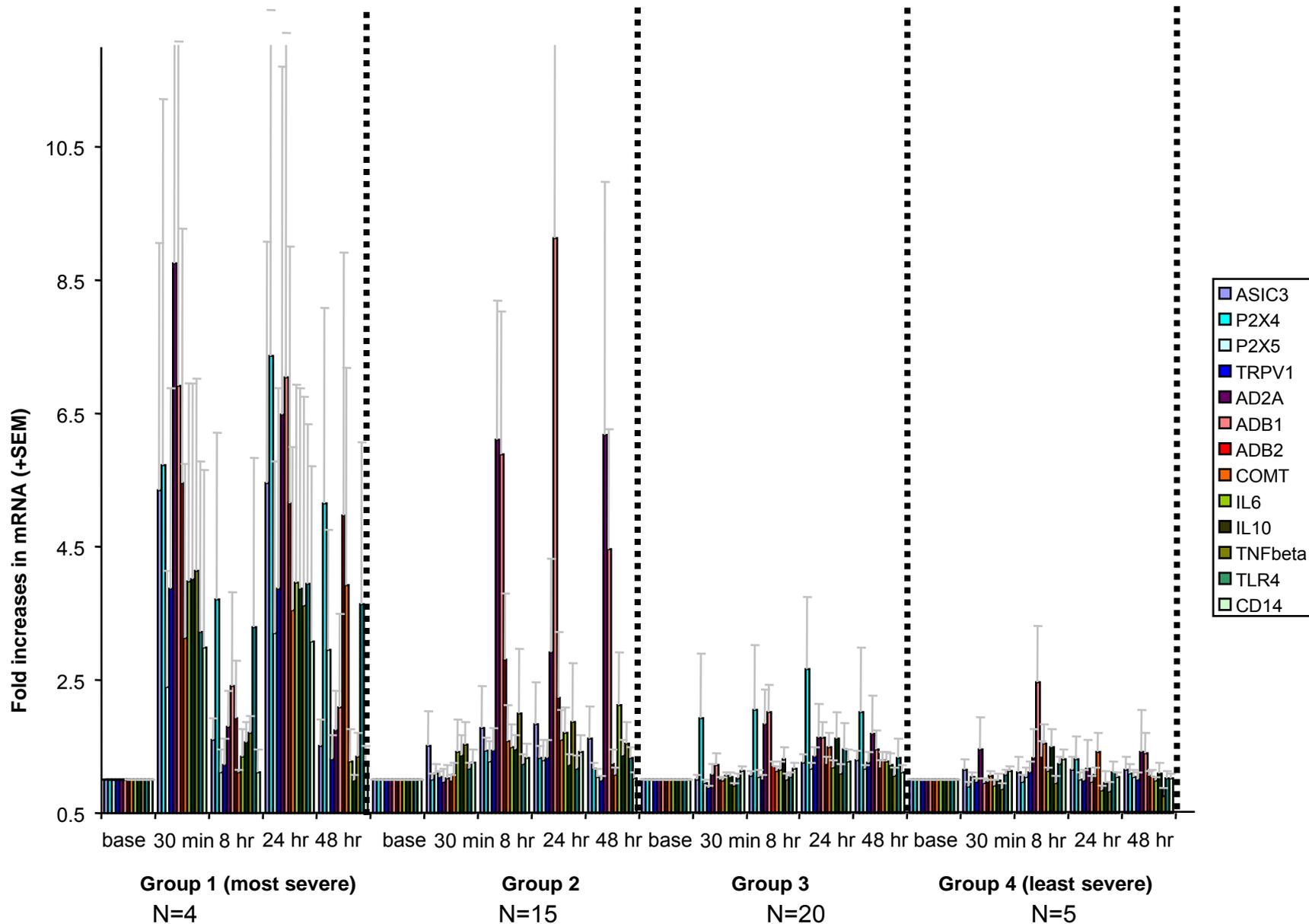
Arrows indicate genes that are different between CFS only and CFS comorbid with FM

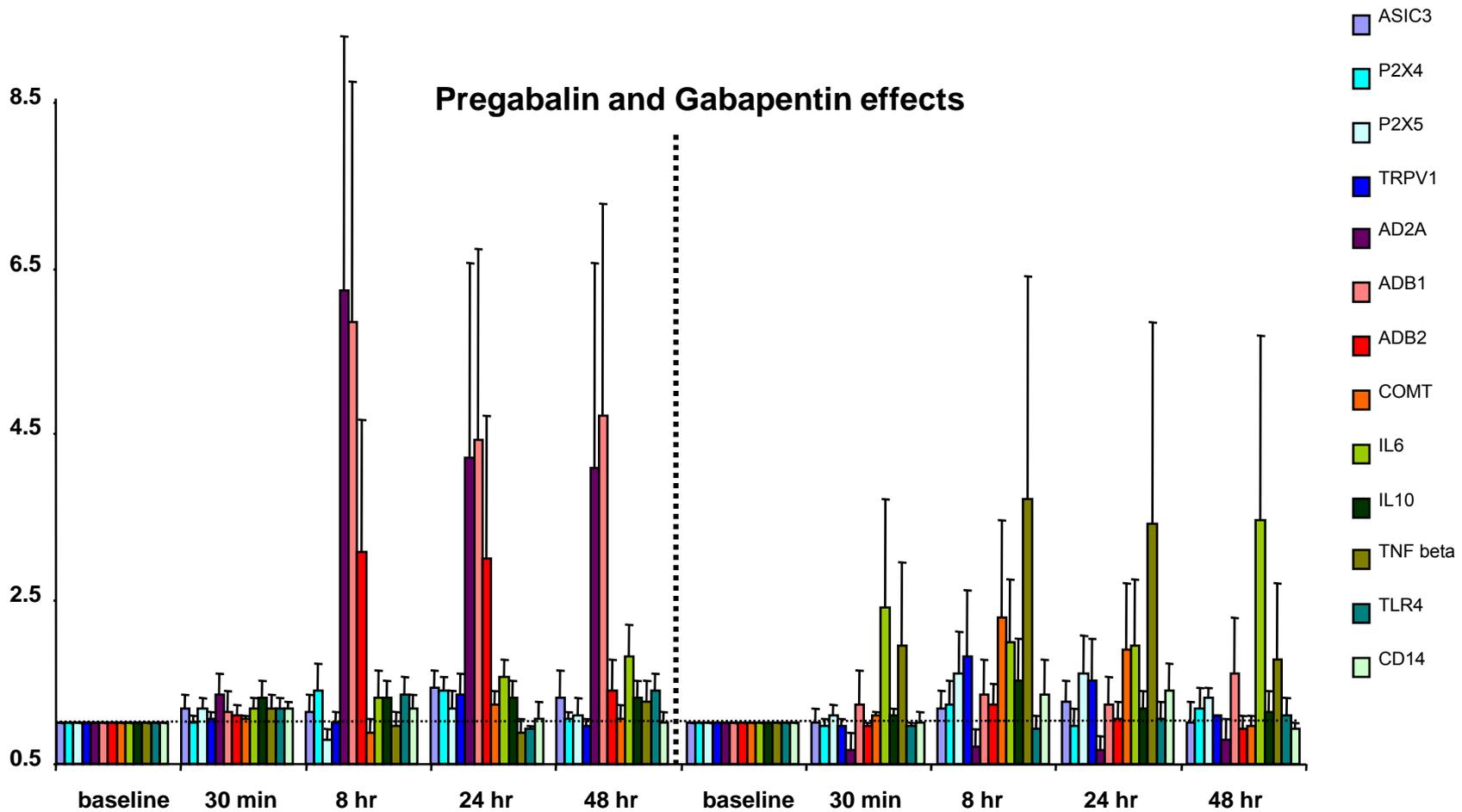


Multiple Sclerosis patients and controls at much higher exercise rates do not show the gene expression increases observed in CFS and CFS+FM patients (compare with previous slide)



Gene expression of CFS+FMS patients sorted by clinical severity (as indicated by degree of disability)

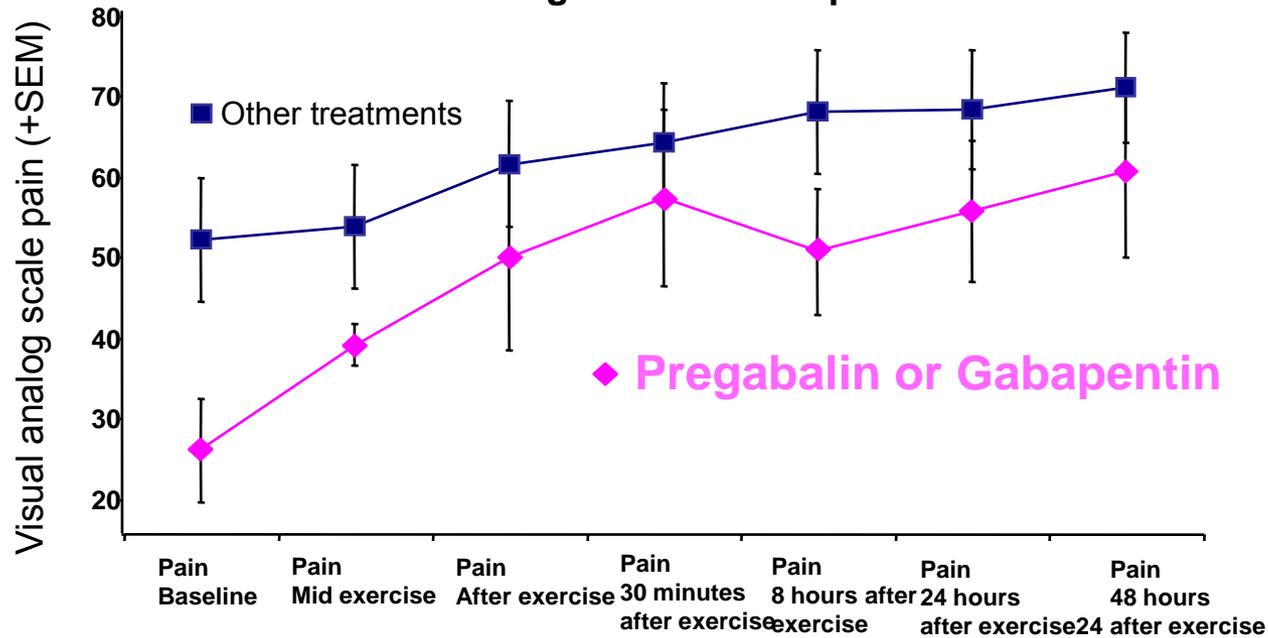




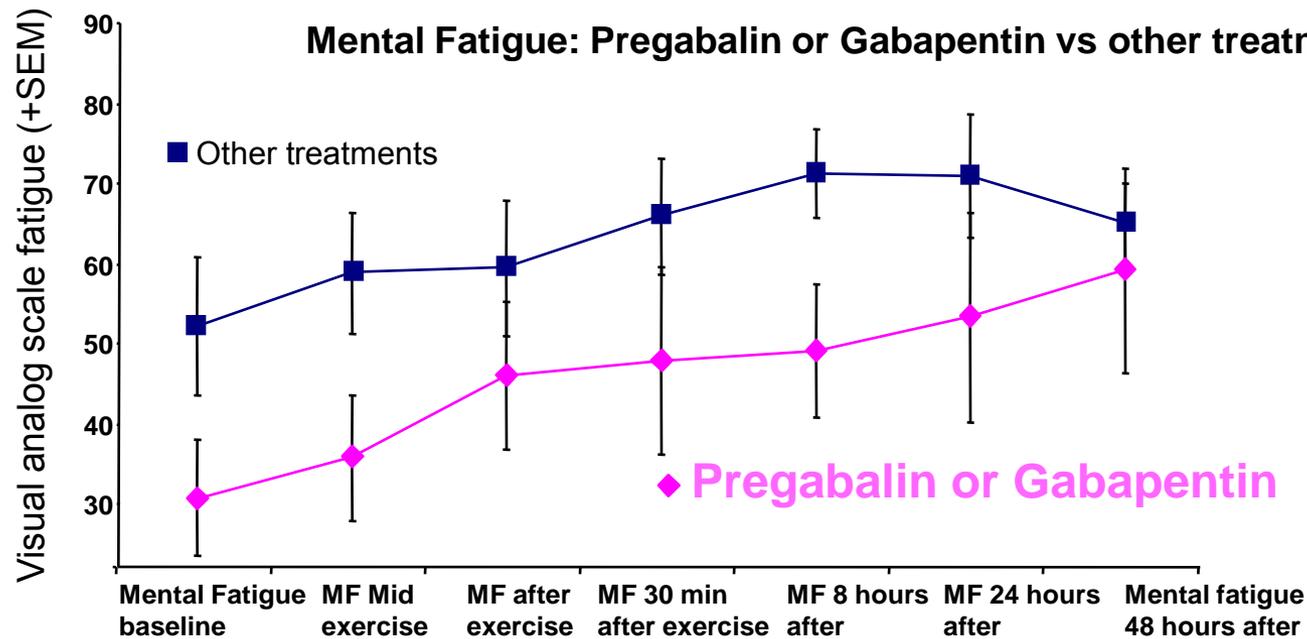
Age, gender, severity patients not on Pregabalin or Gabapentin (n=9)

Patients on Pregabalin or Gabapentin (n=5)

Pain: Pregabalin or Gabapentin vs other treatments



Mental Fatigue: Pregabalin or Gabapentin vs other treatments



Other findings

- Large subgroup based on gene expression
- Age and gender differences
- Combining gene expression with physiological measures can be used to guide treatment
- Utah population data base research by Albright et al. indicates strong familial, likely genetic risk factor for CFS
- Gene expression differences between FM and CFS



Much more to be done

- CDC and Kerr work needs to be followed up with new microarrays to look for transcription factors and expression differences in CFS in both **protein coding** and **non-coding RNA**.
- DNA methylation and histone modifications need analysis in CFS
- Investigation of causes, e.g., viral or bacterial infections trauma, overtraining, genetic predispositions should be conducted along with investigations of the fundamental nature of fatigue



Much more to be done

- Neural pathways for sensory fatigue must be defined
- Objective biomarkers for CFS can, and should be developed and approved for human use
- These markers should be used to guide effective treatment of CFS and CFS+FMS patients, and prevention of CFS in the general population

