DR ALAN LIGHT
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Working on pain pathways for last 40 years
Interested in fatigue about that point too

Genetic and Genomic markers of CFS risk and prognosis

Genomics in CFS
- Almost everybody uses CDC, some use CDC and Canadian
- Quick review of some of previous results done with genetics and gene expression work
- Genomics in CFS – article just came out in May – a meta-analysis review of genetics and epigenetics of CFS.
  - They made a few statements: although altered HPA and association with infectious agents have been identified, the search for standard genetic or epigenetic markers has been relatively unproductive or nonexistent (epigenetic)
  - His caveat – “maybe” – some findings not unproductive
  - 2nd statement – levels of fatigue influenced by genetic or epigenetic –
  - He believes genetics and genomics can define genotype. So the genetics and the genetic expression can improve your definition of the phenotype.
- Studies need to focus not only on SNPs or differential gene expression of disease risk or state but also systems biology, env’t influences, and design of functional studies
- Caveat – need to know the systems in order to study them.
- Limitation of gene data – all info is from leukocytes, or worse, whole blood – can contaminate
- Leukocyte may not be most important – symptoms muscle and brain
- Problem – could examine muscle, but different to obtain brain or sensory material (post-mortem problems of RNA degradation) – then limited to genetic analysis, which can be done different ways.

Genetic association of CFS
- Immune system most likely involved
- Major HLA histocompatibility allele in CFS
- Low interferon gamma
- Implicated immune system
- Changes in HPA Axis – SNPS
- Glucocorticoid receptor

Rajavin et al – also showed SNPS for glucocorticoid receptor – associated with risk for CFS

Serotonergic system

Seratonin receptor associated with CFS

Gene expression association with CFS
- he looks at SNPS, generic changes as causing very little of disease process of CFS
- genetic variation in SNPS very often cause very very small changes (if the person survives).
- Environmental influences with that gene can change the gene much more dramatically This can affect it not just percent but many, many fold.
- Therefore env’t impact can be much larger than genetic mutations
- Can be long-lasting or permanent (epigenetics)

mRNA decreases don’t necessarily reflect changes in proteins they code for
Could be that if your protein product goes down, that may influence gene to turn UP.

mRNA increases informs us not of the gene product itself but the upstream drive... tells us something is trying to change up or down.

Gene expression with CFS
- 25 publications from 6 labs since 2002
- 14 since 2008
- 12 of 25 are from dataset from CDC; many of these are actually very informative
They used the 20K human array for these studies. We now have 44K array for gene arrays.

Associations found: a number of associations, genes that could indicate that did show some interesting genes altered in CFS

Most compact study was done looking @ identical twins. Showed altered HPA and serotonergic immune system. The title of their paper was NO gene expression markers in identical twins! But they did see altered HPA etc.

A good pilot hypothesis:

- all of these systems likely important in CFS

Jonathan Kerr – has done many micro-array studies. Found 88 genes differentially associated with CFS, associated with specific viruses. He has duplicated this – not done externally. Problem – there is no money for replications. Few researchers with capabilities to do these experiments correctly.

Gene expression related to prognosis and xxx – there isn’t any yet. Hopefully we will be able to do these sorts of things. Requires longitudinal studies using gene expression

Gene expression related to severity – worked backwards using severity to find genes. So far no prospective studies.

A DIFFERENT APPROACH:

Almost all of genetic studies have focused on causes of CFS

- The complaint about phenotype and “elephant and blind men”
- Need to go back to beginning: “what is fatigue”
- There came available a way to study fatigue
- Had to define fatigue differently
- Means different things to different people

Scientists – inability to contract skeletal muscle

NOT what CFS patients experience
Instead what CFS patients experience is a sensory experience – overwhelming sensory tiredness. They CAN do a max V02 – ONE time. But they feel fatigued to the point where they can’t do something. Their muscles still contract. Their fatigue can be dramatically increased by a short duration of seemingly mild exercise.

We have postulated a sensory fatigue phenomenon

2 flavors

- SENSORY PHENOMENON - sensory of tiredness, pressure, leaded legness, etc that comes beforehand

- MENTAL PERCEPTION: perception of mental tiredness, confusion, etc

They have been throwing substances at sensory neurons for years to try to find the chemical that causes this sensation of muscle pain and fatigue. Breakthrough – Ed McCluskie showed that in heart muscle he found sensory neurons that were encoding metabolites and the sensory receptors encoded for acid-based ion channels were...

He then discovered that the molecule doesn’t respond well to protons well at all unless you combine it with lactate and ATP – 3 metabolites. What is ATP doing increasing in muscle with exercise? It DOES increase when you contract your muscle. Don’t get the same metabolic buildup from metabolic acidosis... receptors don’t get fooled – they know when they are really contracting.

So Lights used that info to determine all the molecular receptors. They focused on the question of the biology behind the disease. So published in 2008 the ASIC 2-3 heteromeres. Indicates that more than one ASIC receptor could be involved. Since all 3 ASICs are present in humans, could substitute one for another. Found there are 2 classes of sensory neurons

- respond to levels of metabolites that are painful (eg. low pH)
- respond to levels of metabolites that can not be painful

Eg. receptor for heat – there are more of these receptors in muscle than in skin -

Adrenergic Receptors

–discovered that Beta 2 and 1 receptors can enhance metabolite signaling from pain and fatigue receptors.
Found that you can use a low concentration of beta blocker (propanolol) and it will block pain and fatigue in FM and TMJ patients.

Also know that cytokines are important for CFS and FM – these can enhance signals of cytokine receptors – they in fact increase their sensitivity to these metabolites.

Adrenergic receptors UPREGULATED by inflammation.

So can we use this with patients with unexplained fatigue and muscle pain? Am FM association – looking at patients with FM and CFS.

- CFS – determined that all critical genes on muscle sensory neurons are also on leukocytes. Sounds like a huge jump, but maybe not... Leukocytes ARE aware of the detection that your muscle makes....

- They also circulate thru muscle

Protocol
- 25 minutes
- See Journal of Pain
- Canadian and CDC guidelines
- Brought carefully into clinic – not stressed for 2 days
- Exercise; baseline; 24, 28 hrs
- Series of questionnaires

Gene expression changes in CFS
- Asic 3, Tryp 341, etc
- WE looked at only 13 genes that represented sensory, adrenergic and immune system
- No change at baseline
- On exercise – color coded normal control genes on top of graph; no significant change on exercise
- CFS with comorbid FM – Show increase in ion channels up dramatically 5x at just 30 mins after exercise. And these stay up, at least to 48 hours – don’t know when they get down
- Adrenergic receptors in CFS with FM – up again. Beta 1 the most dramatically up. Beta 2 probably the best (red)
- Cytokines – some of these are up significantly – most prominent thing we see is both TNF alpha; IL10, IL6 are up. Both inflammatory and anti-inflammatory up at the same time. However compared to the others, they are up less.

- Looked also at CFS only patients, without comorbid FM – looks pretty much the same. Differences are in ASIC and TRYP1

- Pattern very different with MS patients. This is a different syndrome, and in fact for the MS patient, most is probably due to brain alterations and not so much due to alterations elsewhere.

- Finally, these are high-intensity controls and exercised them to 85% of predicted maximal heart rate. Even with that degree of exercise, there are very few differences from baseline. Some bump-up of cytokine receptors at 48 hrs

- Cindy Bateman their clinician had rated their patients according to severity. Grouped 1,2,3,4 from least to most severe. Most severe on left of graph. Group 2 – majority of genes are less expressed. So they can track severity with this gene special measure.

- Treatment effects: By no means a good study – we’re trying to do the good one now. Trying to look at effect of 3 gabba-pentin – given a trade-name drug vs controls. The genes most related to fatigue and pain are decreased, whereas cytokines are bumped up a bit. So one might see a treatment opportunity – now doing double-blind, placebo-controlled crossover study.

- There was a difference in fatigue AND pain scores if on drug. They actually showed a decreased mental fatigue score and pain score. There were some outliers – some nor affected.

- Goal now to determine whether gene expression measure can be used to predict which patients will benefit from treatment and understand just what is going on

- Because we know these drugs don’t CURE these people, but they do improve symptoms

- We have found a large subgroup that I can’t talk about (pending publication) – if we separate the subgroups a lot of variance goes away.

- Cindy Bateman – each patient has their own pattern – is this primarily an adrenergic phenomenon in a given patient, or a sensory phenomenon...

- We also have another finding – even tho identical twin study didn’t find anything – in fact this is looking at genetic differences. What we found in the database is a strong familial link – likely a genetic risk factor – for CFS

- Cindy Bateman found this genetic factor in at least 1 family – we could work backwards from that.

- We now have – thanks to the American FM association, there are gene expression differences – it will be forthcoming soon. The differences are fairly remarkable.

- Much more to be done

- CDC and Kerr work needs to be followed up
- DNA methylation and histone modification meds need analysis in CFS
- Investigation of causes (eg. viral or bacterial) need to be continued
- Trauma, overtraining, genetics should be conducted along with investigations into the fundamental nature of fatigue (i.e. not just ability to contract muscle)

DR LEONARD JASON, DEPAUL UNIVERSITY, CHICAGO

Differentiating Level 1 and Level 2 Indices

Level 1: Objective (biological/behavioral)

Level 2: Self-report

Implications for assessment, diagnosis, and treatment

How does one do an assessment, what are the core areas that need to be assessed. The two criteria I would talk about – Fukuda; and the Canadian Criteria

- marked degree of nuance

How to operationalize these terms:

- i.e. what does it mean to have substantial reductions in previous educational, social, personal activities. I believe more work needs to be done.

LEVEL 1

- One way to get at fatigue is actigraphy
- Is an objective way to figure out how much activity a person is involved in
- Slide: healthy vs CFS actigraphy. These types of actigraphs can be worn 24 hrs/day x 2 weeks
- Patient with CFS – diurnal patterns are now completely GONE. Not a difference in total amount of activity. (THIS WAS AN AMAZING SLIDE)

LEVEL 2
- Usually relies on fatigue scales
- Many fatigue scales do not accurately reflect severe fatigue of CFS
- Intensity and chronicity – we often fail to measure these.
- Measuring intensity: If you look at fatigue in a healthy person, one out of four people will experience fatigue. It’s not occurrence but INTENSITY of symptoms that are important.
- If one looked @ percent reporting fatigue for 6 mos or longer – there are no differences between the % of these 2 groups. These are different from controls. But differentiating CFS and major depressive – look at % reporting SEVERITY of fatigue... Major depressive is @ 50% on severity; ME/CFS is 80-100 in severity.
- Chicago group is trying to think about fatigue as way Eskimos think about snow. Fatigue – Chicago has 5 ways they think can be manifested:
  - “Wired fatigue” (CFS patients readily reflect this)
  - Brain fog fatigue
  - 3 more factors
- CFS patients are able to differentiate these 5 different types of fatigue.
- ACTRE – timelog – every 30 mins they do ratings. You get 2 days of activity and a profile of functioning. This is a level 2 type of measure of fatigue.
- When looking @ CFS, 75% of time patients experience fatigue; rest 25%; different from MDD (Major Depressive Order)
- Intensity and chronicity in understanding fatigue – we really need to understand fatigue IN RELATION TO ACTIVITY
- Measuring intensity of fatigue – they were engaging in minimal activity, but had maximal fatigue, even tho they had almost no activity. This helps us understand that fatigue needs to be understood by intensity (eg. 0-10), and what happens over time
- Another patient might have variability in their fatigue and activity. This patient is functional, but gets a lot of fatigue that is activity-related.
- Our group is also interested in perceived energy vs actual. This person doesn’t really have a lot of perceived available energy. And yet their expended energy is over their perceived energy – this person is probably overdoing it. When we did that, we were able to see some reductions in their fatigue level. What we are suggesting is this idea of pacing, staying in an energy envelope – important clinical implications. Try NOT to get into post-exertional state.

Quick intervention of what can be done with these findings
randomly assigned individuals to buddy or control

- Student buddy came to help individual reduce energy expenditures by helping out with some activities

- Found significant reduction in fatigue and increases in vitality

- Suggests Level 2 variables can be useful to understand changes over time in patients.

- One type of clinical intervention – pacing, staying within energy envelope

**Fatigue causing disability**

- MOS or SF36 are commons ways to measure functional issue of fatigue impacting function

- Buchwald and others have looked at CFS, distinguishing from other fatiguing illnesses

- Suggestion that Role-Emotional subscale has poor specificity/sensitivity for identifying patients with CFS

- Vitality, Social Functioning and Role Physical – highest specificity/sensitivity

Some patients might to learn to control their fatigue but they continue to lack stamina/endurance – they’re still incredibly incapacitated

- Can we elicit core symptoms by pushing them in effect

**Post-Exertional Malaise- could be the CRITICAL symptom/key of this illness**

Must consider if PEM is critical – do we want to make it a requirement (Canadian) instead of optional (Fukuda)

**Level 1 can also be done by cardiopulmonary testing; anaerobic threshold. Can also look at Max V02**

**Other Level 2 measures**

- Do you get heavy feeling after exercise?

- Drained or sick after exercise?

**SLEEP**
- Canadian – unrefreshing sleep; or rhythm disturbance; high frequency of sleep disorders in CFS and FM, with alpha intrusion – tho seen also in other chronic condition
- Level 1 – can bring pt in for lab study of sleep
- Level 2 – self-report of sleep quality – eg. Pittsburg sleep questionnaire

PAIN
- Canadian Criteria – significant arthralgia and/or myalgia
- Fukuda – headaches; muscle; throat; lymph nodes;
- Level 1 pain – use types of methods that Lights suggested for biological documentation
- Level 2- used thru the McGill Pain Questionnaire

NEUROCOGNITIVE
- confusion
- impairment of concentration
- short-term memory
- Level 1 documentation: MRI, SPECT, PET indicating reductions in gray matter – declines linked to reduction in physical activity
- Level 2 – CANTAB test

AUTONOMIC AND/OR

NEUROENDOCRINE AND/OR

IMMUNE MANIFESTATIONS
Level 1 Autonomic – Tilt Table – dec BP, inc HR
81% of patients with CFS experience ejection fraction decreases, suggesting ventricular heart problems
Plasma volume and total blood volume – usually low
Tilt table – normal person – 10-15 beat increase
CFS – 30 for initial increase
LEVEL 2 MANIFESTATIONS

DePaul Symptom Questionnaire – eg. feeling unsteady on feet, fainting

Level 1 – Neuro-endocrine

HPA axis dysregulation. Has been finding changes in total antioxidant power, in terms of plasma cortisol and DHEA – manuscript soon to be published

Level 2 – Hot/cold, nightsweats

Level 1 – Immune
- eg. XMRV/MLV’s
- bacteria
- Opportunistic infections
- Over-activation immune system; Th1 – Th2 shift

Level 2 – Immune
- Self-report using DePaul – eg. feverish, having sore throat

Intervention with Immune and Neuroendocrine Biomarkers
- 100 pts non-pharma intervention
- Patients involved with nurse for biweekly meetings
- Improvers vs non-improvers
- A mix of level 1 and 2 measures gives interesting results to measure interventional results
- Two groups at baseline were quite comparable at baseline. BUT one group improved, one did not
- What differentiated them?
- Patients with more severe immune characteristics – the non-improvers
- Pts with Th2 immune response tended not to improve in this particular study
Another marker: normal vs abnormal cortisol markers. Patients baseline cortisol – those with normal baseline cortisol tended to improve. Differential outcomes suggest that patients with normal baseline cortisol IMPROVE. In other words, pts most impaired in HPA axis were least able to improve when provided non-pharmacological interventions.

Some pts were able to keep within energy envelope – others not. Over time, those who were able to stay within their energy envelope had significant reductions in severity. Those not able to got worse over time. Maybe there are common elements in those who improve function (he is careful NOT to say CURE). NB very small % of patients completely recover from this illness.

Findings suggest that helping pts helping maintain energy quotients may help improve functioning over time.

RECOMMENDATIONS

- Advantages to Level 1 and Level 2
- Level 1 has clear advantages
- Level 2 clearly have recall bias problems
- Level 1 are clearly stronger objective measures for intervention, assessment, and case definitions

Also Level 1 and Level 2 there is internal variations of reliability and validity. Eg. activity log of measuring activity every ½ hr was single best predictor when we tried to differentiate MDD from CFS from controls (MDD = major depressive disorder). So even within level 2 timelogs, you get different ways that some might be useful.

Final set of recommendations:

- Sample selection is critical
- Some of the diverse findings not only with XMRV but with prior research may very well have to do with different samples that diff populations have.
- It is possible that PEM is THE critical symptoms
- If we’re recruiting patient samples without this critical symptom, we may not be having individuals with this syndrome. The reason is that patients who have learned to cope with the illness
may have low fatigue, but zero life (my summary). BUT these patients still could be given entry to our trials if we thought of the importance of PEM (as pathognomic).

There is high variability of illness manifestations of this disorder. The only way to get around this is to get large databases. That allows for the specification of different subtypes – and then can develop appropriate (targeted) treatment.

DR MARTIN LERNER

I’d like to credit my co-authors here. (see co-authors on slide 1)

- Over the years, and this has been an activity of over 20 years, there has been significant help from co-authors and investigators, some of whom listed here

- CFS Chart study – 142 patients from 2001 – 2007 in same CFS treatment centre; each pt receiving treatment for at least 6 mos. Remarkable finding...

- Discussion of critical measure of how “sick” our patients are. Can be done at bedside by office dr and CFS patient at bedside. Is validated by accepted fatigue scores.

- I must say I began an unexpected investigative infectious diseases career that completely changed my life for which I am tremendously grateful. As other infectious disease people I was totally uninterested in CFS until I became overwhelmingly ill in 1988. I had chest ache. I thought I had a heart attack. I had 24 hr ECG. I had an acute dilated cardiomyopathy of unknown cause. From 1988 – 1996...in 1996 I was the first person treated as I shall outline today.

- Our study began in 2001 because at that time Valcyte (oral) could be replaced with gan(ciclivor?)

- Means of analysis: Initial selection; data collection; analysis

- All patients in our group had one or several IgG titres

- IgG titres are the stable titre of experience and not the early titre of IgM which says an infection is being responded to.

- The severity of illness was based on what a doctor could determine at the bedside. Readily available score.

- Group A and B – viruses

- Group B had co-infectoins with Lyme (tick-borne diseases)

- Group A and B totally indistinguishable clinically.
- All meet Fukuda criteria for CFS and the result of that simple statement, no clinical trial will show activity

- To get ahead of our story, we found that EBV was in 30% of our patients - singly without any other infection; and in 81% of our group A patients otherwise.

- Importantly studies for Borellia Burgdorfe. Importantly the studies that we used utilized the antigens used by the CDC. Adult rheumatic fever looks indistinguishably like CFS. And after we were finished we realized some pts had grossly elevated mycoplasma pneumonia titres in addition to EBV etc.

- We did 24 hr holter monitors and 85% had grossly abnormal Holter monitors

- First paper using Holter monitor showed grossly abnormal electrical recovery of the heart, repolarizations, inversions, flattenings

- Cardiologists know nothing about heart muscle disease which is a basic part of CFS.

- The Energy Index Point Score – Functional Capacity Criteria

- I DO NOT advise exercise until they are able not to nap during the day.

- Many patients complain of many, many symptoms. These symptoms have been spoken about elegantly today.

- As the Energy Index Point Score improves, symptoms decrease and disappear. We measured 22 healthy patients and 20 CFS patients. The EIPS is quick, easy, dependable... provides a validated understanding of where your patient is – and it has been translated

- Group A patients
  - EBV – treated with Valacyclovir. Amount of drug in blood had to be greater than the IV-50, the infectious dose 50 for 85% of the day – dosage calibrated accordingly
  - HCM/HHV-6 treated with Valganciclovir

- Group B
  - EBV treated with Valacyclovir as in Group A
  - HCMV/HHV6 treated with Valganciclovir as in Group A (HCMV = cytomegalovirus)
  - Coinfections treated with antibiotics
  - Mean age 45,46. No other diseases. Well until they became ill with CFS. Mean duration of CFS was almost 5 years and that’s important because the shorter the period of illness, the easier it is.

- 74.5% Group A responders; several successive cohorts
- Regularity of increases were so regular, chances of them being by chance are less than 1 in 1,000 (p< .001)
- If one looks at the responders vs nonresponders, they are not different in age, BMI, but remarkably different in how long they were ill before starting treatment. ~7 yrs for nonresponders
- Response NOT different for single vs multiple viruses
- Mean EIPS baseline:: 4.3 to 6.8 at end
- Cardiac, neurologic, neuro-cognitive abnormalities improved and/or disappeared

Difference between non-responders and responders – sicker longer

24 hr Holter Monitor – T waves upright in 2nd ... every one tested for coronary artery disease. If you look at the myocardial dynamics of these patients – they were frightening. The heart contracts in a symmetrical wave. When they contracted the heart it expanded. Definitely a cardiomyopathy. Their EF’s (ejection fractions) were decreased. This is a biopsy of one of the hearts. The blue is scar tissue. The myocardial fibers are in disarray.

Herpesvirus image
- outer coat – double layer of lipid
- glycoprotein spikes
- Antigen that we measure – structural antigen is the only measure of the virus we see. We make no antibodies to these proteins
- This is also an exciting slide which allowed me to go on all these years. It indicates the viral infection. This reflects an OLD infection and here we have integument proteins. About the 50th gene... there are 200 genes. There are early to late. Xxx which are only present in CFS patients – disappeared.

This slide is from a manuscript that is now under editorial review. Permissive and non-permissive replication. In active infection one sees, travelling in the circulation EBV, HHV-6, CMV, etc... Antigen is present in the blood in permissive infection. Ig antibody is present in permissive infection. Immediate early gene products are often present in permissive infection and the antiviral agents that we use inhibit the DNA polymerase and inhibit the virus. BUT in CFS it’s all different. There IS Ig antibody to integument proteins. There is Ab to immediate early antigen. There is immediate early antigen to CMF and we predict early antigen to HHV-6. And we also note it took 6 months for our patients to begin to get well. The reason for this delay – the antiviral effect in permissive replication is swift but in CFS is slow.
If one reads the virology texts one recognizes Herpesviruses as having biphasic... means the host cell plus the virus enter and either destroy the cell with permissive infection and necrosis. Or it enters the cell. Enters the nucleus. And in the case of EBV and CMV is a non-integrated episome staying for the whole life (if pt remains well). What we hypothesize – and have a great deal of evidence to support – is that immediate early antigen, dysregulates the cell, induces all the changes that Nancy Klimas recognizes, the cell dies (apoptosis which is recognized pathologically). The immediate early antigen known as Early Gene ZTA, induces apoptosis and destruction of the cell. New virus is NOT formed.

Conclusion

1) Antiviral nucleosides valacyclovir (EBV) and valganciclovir (HCMV, HHV-t, inhibitherpes host=cell necrosis and host-cell apoptosis

2) Causal relationship between CFS and eBV/HHV-6, HCMV, specifically nonpermissive replication producing host-cell apoptosis

3) Previous research has not proven antiviral success due to limited timelines and the lack of CFS classification.

4) Long-term group and subset-directed antiviral treatment is successful

5) Patients returned to their lives.