

Gene Variants, Mitochondria and Autoimmunity in ME/CFS and Fibromyalgia

Alan Light, Ph.D., Kathleen Light, Ph.D. and Lucinda Bateman, M.D.
Dept. of Anesthesiology, University of Utah School of Medicine
Bateman Horne Center for ME/CFS & FM

Supported by grants from:
Dept. of Anesthesiology
U of U School of Medicine
NIAMS, NINDS, NHLB I
SolveCFS and AFSA
Generous patient donors

Disclosures

Our group has disclosed information on autoimmune and gene variants as potential biomarkers for CFS and FM to University of Utah TVC

TVC has also applied for patents based on our data showing that RNA alterations in parts of the fatigue pathways may be used as biomarkers for CFS and FM



ME/CFS and Fibromyalgia

- Myalgic encephalomyelitis or chronic fatigue syndrome (ME/CFS) is a health problem affecting at least 1 - 2 million adults in the USA and at least 1-2% of the population worldwide including children (Jason et al, 2006; webpage NIH, 2017).
- Characterized by severe physical fatigue, mental fog, muscle and joint pain and post-exertional worsening of symptoms, ME/CFS is so debilitating that at least 25% of sufferers are classified as disabled or unemployable (Ross et al, 2004).
- Fibromyalgia syndrome (FM), characterized by persistent widespread pain with fatigue being a frequent secondary complaint, may affect 2-3 times as many patients as ME/CFS. In up to 70% of cases, ME/CFS patients also meet clinical criteria for FM (Aaron et al, 2000; Kato et al, 2006).



ME/CFS and Fibromyalgia

- Our first research efforts were aimed at determining what caused some of the major symptoms of ME/CFS
- We started with determining what causes the sensations of fatigue. We determined that signaling from neurons that innervated muscle caused the sensations of fatigue caused by exercise.
- We further determined that combinations of metabolites that are produced during exercise activate the fatigue signaling neurons, and that these same metabolites in higher amounts could cause aching pain.



ME/CFS and Fibromyalgia

- We then used gene expression to determine that fatigue signaling in patients with ME/CFS and/or Fibromyalgia was greatly altered in a way that could cause the symptoms of fatigue and muscle pain.
- However, it was obvious that the cause of ME/CFS was not fatigue itself.
- The fact that our gene expression was from immune cells suggested that immune function might hold clues to the causes of ME/CFS and Fibromyalgia.



Autoimmunity and Mutations in ME/CFS

- Three recent publications indicated that autoimmune disease may cause ME/CFS in at least a subgroup of patients. Two of these papers suggest that it might be treatable with drugs that alter autoimmune responses.
- We recently collaborated with the Oklahoma group (M. Cunningham) to extend these findings on autoimmunity. We also began exploring whether ME/CFS patients have gene variants that make them more susceptible to autoimmune responses or to decreased cell energy (mitochondrial mutations) or both of these in combination.

Autoimmunity and Mutations in ME/CFS

- **#1. Autoimmune Basis for Postural Tachycardia Syndrome**
 - Li, Cunningham, et al. JAMA 2014; 3: e000755
- **#2. B-Lymphocyte Depletion in Myalgic Encephalopathy/ Chronic Fatigue Syndrome. An Open-Label Phase II Study with Rituximab Maintenance Treatment.**
 - Fluge Ø, et al. (2015) PLoS ONE 10(7): e0129898.
- **#3. Antibodies to β adrenergic and muscarinic cholinergic receptors in patients with Chronic Fatigue Syndrome**
 - Loebel et al Brain, Behavior, and Immunity (October 2015)



Our Pilot Study on Autoimmunity in ME/CFS

- We sent the Oklahoma group plasma samples from **18 ME/CFS patients with either POTS or orthostatic intolerance** (can't stand up for 10 min without nearly fainting). **14 of 18 had comorbid FM.**
- They did blinded multiple autoimmune assays including Beta-1 and 2 adrenergic and M2 acetylcholine receptors. As a second test of beta adrenergic receptor autoimmune activity, protein kinase A (PKA) responses, a key regulator of T and B cell immune responses, were tested in the presence of beta adrenergic agents.



Our Pilot Study on Autoimmunity in ME/CFS

Results:

- Altogether, 15 of 18 ME/CFS patients with POTS or orthostatic intolerance (83%) had positive autoimmune findings to Beta-adrenergic receptors/PKA or M2 receptors ($P < .001$ vs. controls).
- While these auto antibodies could cause the symptoms seen in these patients, the question is, why do these patients make these auto antibodies?
- Could a mutation in some other gene be involved?



Our Pilot Study on Gene variants in ME/CFS+FM Using RNAseq

- RNAseq sequences the RNA of specific tissues. In our study, the tissue was white blood cells (immune cells) from patients with ME/CFS and/or Fibromyalgia
- This method can find both genetic (inherited) and somatic (acquired) mutations in these white blood cells.



Our Pilot Study on Gene variants in ME/CFS+FM

- Last year, we presented some very preliminary results using RNAseq to this group. Just recently, we finished analysis of both the gene expression differences and possible genetic and somatic mutations found in CFS and FM patients.
- Gene variants were examined in 40 patients with ME/CFS and/or FM. Of these, 14 were the same ME/CFS patients with high autoantibodies on prior tests.
- There were 31 controls.
- We first looked for variants in mitochondrial DNA (separate from 23 chromosomes) because the mitochondria are the energy source for all cells in the body, and they also influence autoimmune responses.

What's the connection of mitochondria to ME/CFS and FM?

- What are mitochondria? Mitochondria are often called the 'cell's powerhouse.' They are specialized compartments within almost every cell. They are responsible for producing 90% of the energy needed by our body to sustain life. Mitochondria combine oxygen from the air we breathe with calories from food to produce energy (stored as ATP).



What's the connection of mitochondria to autoimmunity?

- What is mitochondrial disease? Mitochondrial diseases result when there is a defect (often from a genetic mutation) that reduces the ability of the mitochondria to produce energy. Because the mitochondria fails to produce enough energy, the cell will not function properly.
- Several autoimmune diseases (lupus, rheumatoid arthritis, multiple sclerosis) appear to have a mitochondrial contribution.
- Somatic mutations in mitochondria (acquired later in life rather than inherited) can trigger and/or sustain T and B cell responses in autoimmune diseases. Mutations are roughly 15 times as common in mitochondrial DNA vs. chromosomal DNA, and somatic mutations are much more common.

Gene variants in Our Pilot Sample of CFS+FM

- All but 1 of the CFS and FM patients had at least one mitochondrial gene variant (mutation) that was not seen in healthy controls.
- Most of them (more than 70%) had a variant with **High** or **Moderate** Impact in disruption of a key mitochondrial protein.
- **High impact**= where the sequence to be copied is cut short due to adding a stop codon or where part of the sequence is shifted to the wrong section of the gene. **Moderate impact**= missense variant where length is preserved but at least 1 amino acid is incorrectly copied.



The Multiple Variant Hypothesis

More than one hit necessary?

- While a single High or Moderate Impact SNP in a mitochondrial gene may be sufficient to convey susceptibility to ME/CFS and FM, having additional mitochondrial or chromosomal mutations may tip the balance and lead to clinically significant fatigue and pain symptoms (synergistic effects).
- **ME/CFS+FM patients had multiple mitochondrial variants** (from 2-13 variants) affecting mitochondrial Complex I, III, IV and V genes. Complex I and III mutations are linked to mitochondrial inefficiency in producing ATP (energy) and over-production of Reactive Oxygen Species (ROS).



3 ME/CFS+ FM Patients showed the same combination in 5 of 6 mitochondrial mutations

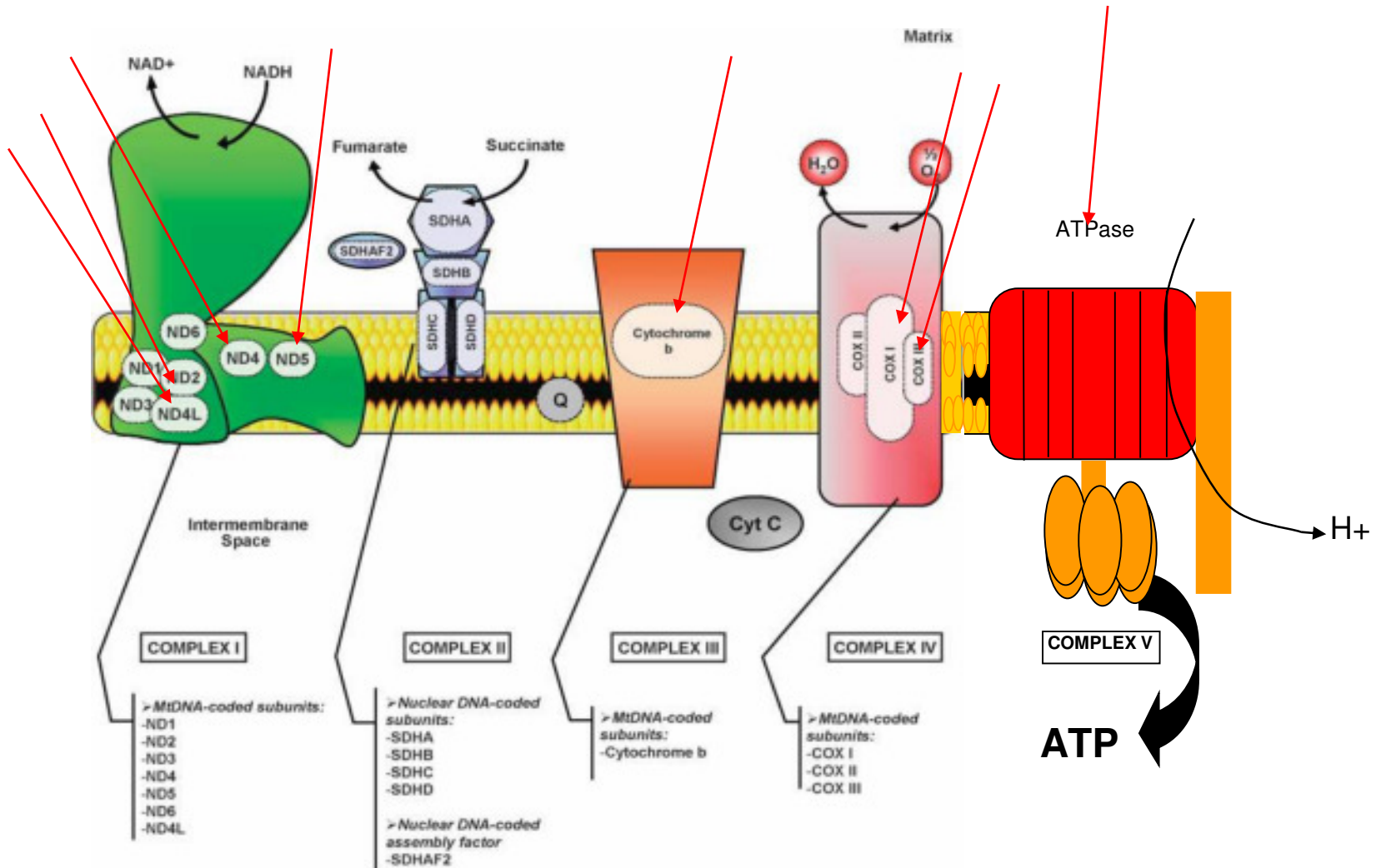
- Gene: MT-ND5 (Complex I) Impact: **High**, stop gained
-
- Gene: MT-CO1 (Complex IV) Impact: **Moderate**, missense
-
- Gene: MT-CO1 (Complex IV) Impact: **Moderate**, missense
-
- Gene MT-ND1, ND2, TQ (Complex I) Impact: **Low**, Modifiers
-
- Gene MT-CYB (Complex III) Impact: **Low**, Modifier
-
- Gene: MT-ATP8 (Complex V) Impact: **Moderate**, missense in 1 patient, and Gene MT-ND1, ND2, TQ (Complex I) Impact: **Low**, Modifiers in 2 patients.



1 ME/CFS Patient showed 11 mitochondrial mutations

- 1. Gene: MT-ND4L Impact: **Moderate**, missense variant
 - 2. Gene: MT-ND5 Impact : **Moderate**, missense variant
 - 3. Gene: MT-CYB Impact: **Moderate**, missense variant
 - 4. Gene: MT-CO1 Impact: **Moderate**, missense variant
 - 5. Gene: MT-CO3 Impact, **Moderate**, missense variant
 - 6. MT-ATP6, CO3, ND3,4, 4L, CYB Impact: **Low**, Modifier
 - 7. Gene: MT-RNR1,2, CO1, ND1,2 Impact: **Low**, Modifier
 - 8. Gene MT-CYB Impact: **Low**, Modifier
 - 9. Gene MT-CYB Impact: **Low**, Modifier
 - 10. Gene MT-RNR1,2, CO1, ND1,2 Impact: **Low**, Modifier
 - 11. Gene: MT-ND2 Impact: **Low**, Modifier
-
- Only 1 control had 1 of these mutations

Respiratory chain complexes I through V with MtDNA components identified for each complex. **Red arrows** indicate components with variants in patient from previous slide. ND=NADH dehydrogenase, COX= cytochrome C oxidase.



Confirmation of Decreased Mitochondrial Complex I-IV Proteins in 5 FM Patients

(Benito Sánchez-Domínguez et al 2015)

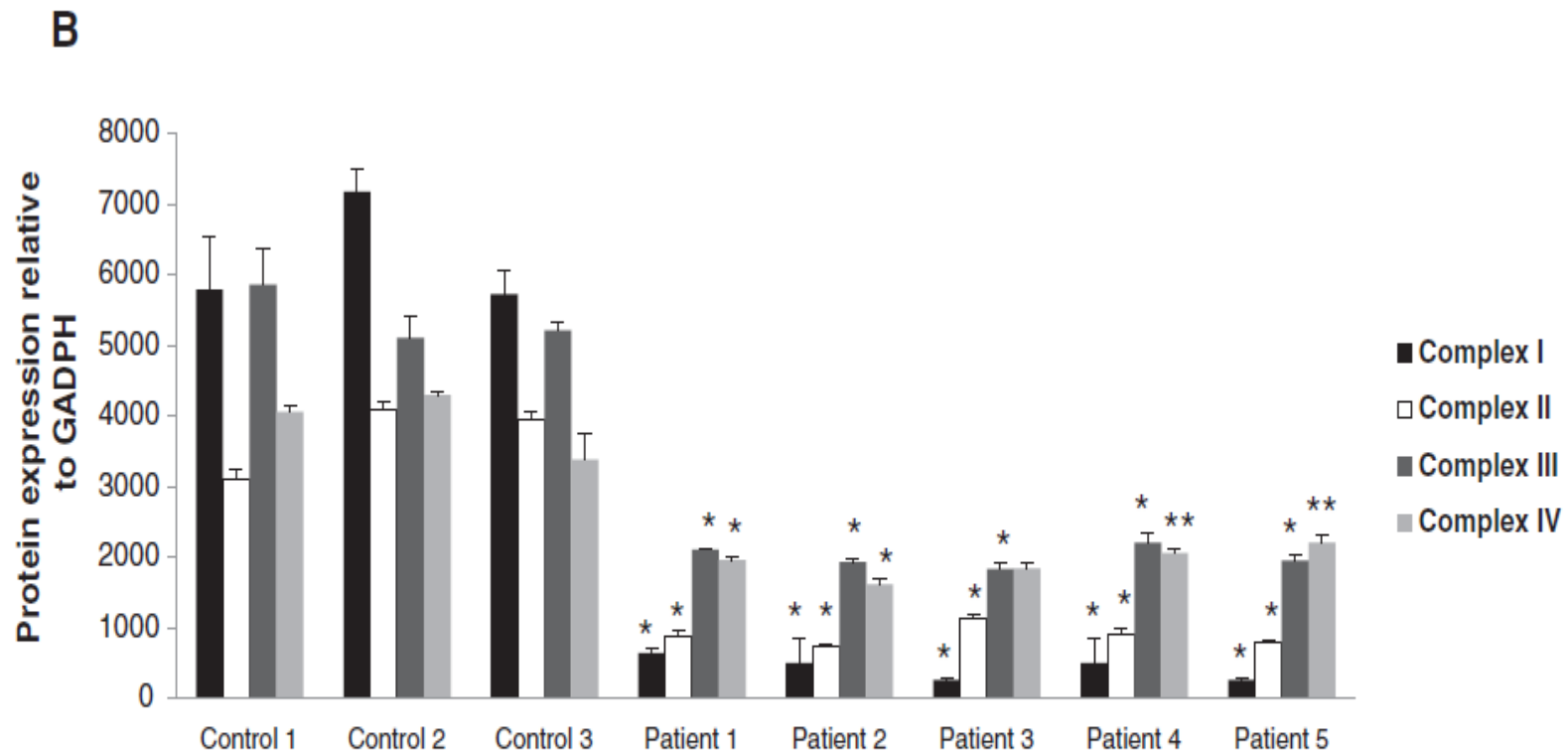


Fig. 1. Mitochondrial chain dysfunction in skin from FM patients ($n = 25$) compared to controls ($n = 20$). (A) Mitochondrial enzymatic activities were determined as described in [Material and methods](#). Results (mean \pm SD) are expressed in U/CS (units per citrate synthase). (B) Protein expression levels of mitochondrial complex I, II, III and complex IV. Protein levels were quantified by densitometric analysis (IOD, integrated optical intensity) of three different Western blots and normalized to GAPDH signal, using skin biopsies from four representative FM patients, compared with three representative healthy age- and sex-matched control subjects. * $P < 0.001$; ** $P < 0.01$ between control and FM patients.

A second group of 7 ME/CFS+ FM Patients showed overlapping mitochondrial and autoimmune mutations

- 1. All had MT-CYB (Complex 3) **Moderate**, missense
- 2. 5 of 7 had 1 mutation each and 1 had both MT-ND6 mutations (Complex 1) **Moderate**, missense
- 3. Same 5 of 7 had MT-ND4L and MT-ND4 mutations (Complex 1) **Moderate**, missense
- No control had any of these variants

- These same patients had variants in autoimmune HLA genes.
- 1. 3 patients had Chr6 HLA-C **Moderate**, missense and 1 of these had a 2nd HLA-C **Moderate**, missense mutation
- 2. 1 had nearby Chr6 HLA-C **Moderate**, missense mutation
- 3. 3 patients had Chr6 HLA-B **Moderate**, missense mutation
- Only 1 control had any of these variants

The Multiple Variant Hypothesis

Many patients had both mitochondrial and autoimmune mutations

- Roughly 80% of ME/CFS+FM patients show mutations in specific autoimmune genes. However, the specific genes were different in each patient.
- All of the ME/CFS+FM patients who showed altered autoimmunity to Beta-adrenergic, M2 acetylcholine receptors or PKA also showed mitochondrial variants.



What's the connection of mitochondria to autoimmunity in our pilot sample?

- We hypothesize that both decreased ATP (energy) from mitochondrial dysfunction AND autoimmune responses are contributing causes of ME/CFS and FM in at least a subgroup.
- Thus, the combination of multiple mutations in several mitochondrial genes OR 1-2 mitochondrial mutations PLUS mutations in autoimmune modulating or other key chromosomal genes may be necessary to cause ME and to identify unique biomarkers for patients with CFS+FM.



Summary and Interpretation of Our Pilot Findings

- Of 18 ME/CFS patients with POTS or orthostatic intolerance (including 14 with comorbid FM), 15 showed a positive autoimmune test, primarily indicating gain of function (up-regulation) of autonomic receptors (beta-adrenergic and cholinergic).
- This would tend to: 1) increase HR, 2) enhance vasodilation in extremities and reduce brain blood flow during upright posture, worsening POTS and orthostatic intolerance, 3) dysregulate normal hemodynamic responses to exercise.
- These same ME/CFS+FM patients with positive autoimmune tests also showed mutations in mitochondrial genes that play important roles in the 5 mitochondrial respiratory complexes that produce 90% of the body's energy. Mitochondrial mutations may also cause altered autoimmune responses.



Summary and Interpretation of Our Pilot Findings

- We suggest that multiple mitochondrial mutations and/or chromosomal mutations create susceptibility to ME/CFS and FM.
- Then, due to exposure to infection inducing a normal immune response where the pathogen may be close enough to our own receptors to cause them to be similarly attacked (molecular mimicry), an autoimmune response is initiated. In some ME/CFS patients, the targets are adrenergic or acetylcholine receptors . In others, targets may be different (endocrine, neural, mitochondria, etc.).
- Thereafter, additional pathogen exposure, or physical or psychological stressors can intensify both the mitochondrial energy deficits and this autoimmune response, creating the cyclical worsening of fatigue, mental fog and pain.



What is next?

- Supported by our New R01 grant from NIH, we plan to test 150 more patients including some with FM alone, with ME/CFS alone and with both ME/CFS+FM. We need to test more volunteers to determine **if the autoimmune and/or mitochondrial genetic variant findings are the same or different in these subgroups of ME/CFS and FM patients**. We also will compare variants to those in otherwise healthy patients with depression or migraines (since many ME/CFS and FM patients have these disorders too). We also need healthy controls (bring your significant other or a friend).
- We also need to expand our autoantibody tests to see **if other ME/CFS and FM patients show different forms of autoimmunity** attacking cells other than adrenergic or muscarinic receptors. (Planned NIH grant)



What is next?

- In addition to more trials with Rituximab and other drugs to limit autoimmune activity, we also need clinical trials to examine the benefits of propranolol and midodrine for patients with autoimmunity-induced gain of beta-adrenergic receptor function and loss of alpha-adrenergic receptor function (Akiko's current and planned NIH grants).



Is mitochondrial dysfunction relevant to symptoms of ME/CFS and FM?

Mitochondrial disease can affect any organ of the body and at any age. Symptoms are extremely diverse and often progressive. They include: muscle weakness, pain in muscles and joints from lactic acidosis, gastrointestinal disorders, swallowing difficulties, susceptibility to infections, strokes, seizures, cardiac disease, liver disease, diabetes, blindness and deafness.

Imagine a major city with half its power plants shut down, with large sections of the city working far below optimum efficiency. Now imagine your body working with one-half of its energy-producing facilities shut down. The brain may be impaired, vision may be dim, muscles may twitch or may be too weak to allow your body to walk or write, your heart may be weakened, and you may not be able to fight off infections. This is precisely the situation of people with mitochondrial disease.

(From United Mitochondrial Disease Foundation web page)

THE END

Thanks to my colleagues and our
wonderful study participants!



1 ME/CFS Patient showed 11 mitochondrial mutations

- 1. M:10507 Gene: MT-ND4L Impact: **Moderate**, missense variant
 - 2. M:14140 Gene: MT-ND5 Impact : **Moderate**, missense variant
 - 3. M:15455 C Gene: MT-CYB Impact: **Moderate**, missense variant
 - 4. M:6519 Gene: MT-CO1 Impact: **Moderate**, missense variant
 - 5. M:9267 Gene: MT-CO3 Impact, **Moderate**, missense variant
 - 6. M:13935 MT-ATP6, CO3, ND3,4, 4L, CYB Impact: **Low**, Modifier
 - 7. M:3012 Gene: MT-RNR1,2, CO1, ND1,2 Impact: **Low**, Modifier
 - 8. M:16303 Gene MT-CYB Impact: **Low**, Modifier
 - 9. M:16345 Gene MT-CYB Impact: **Low**, Modifier
 - 10. M:2296 Gene MT-RNR1,2, CO1, ND1,2 Impact: **Low**, Modifier
 - 11. M:4704 Gene: MT-ND2 Impact: **Low**, Modifier
-
- Only 1 control had 1 of these mutations, M:14140

Our Pilot Study on Autoimmunity in ME/CFS

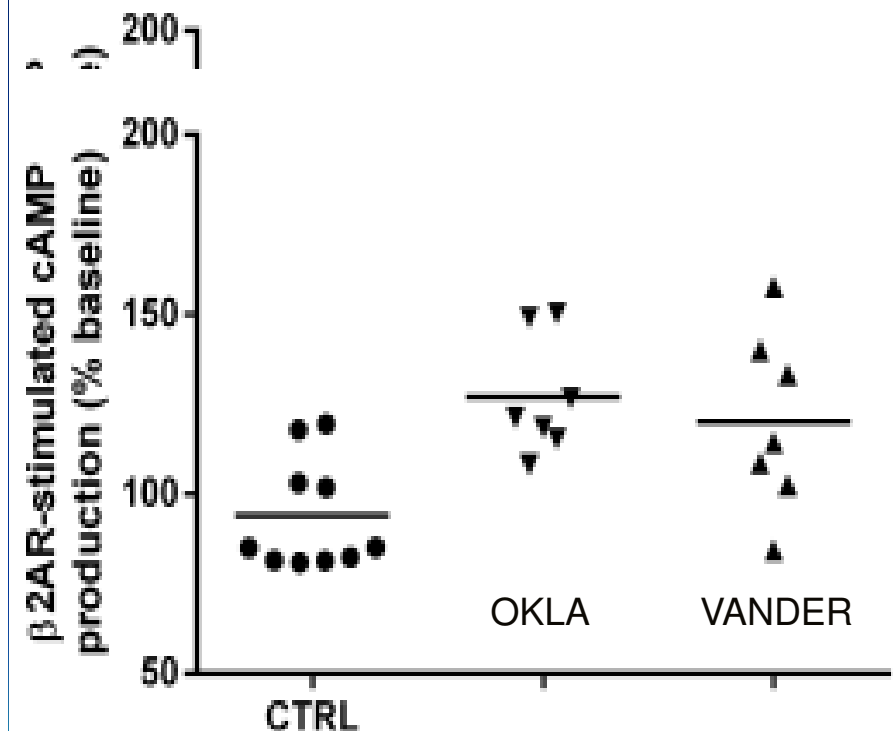
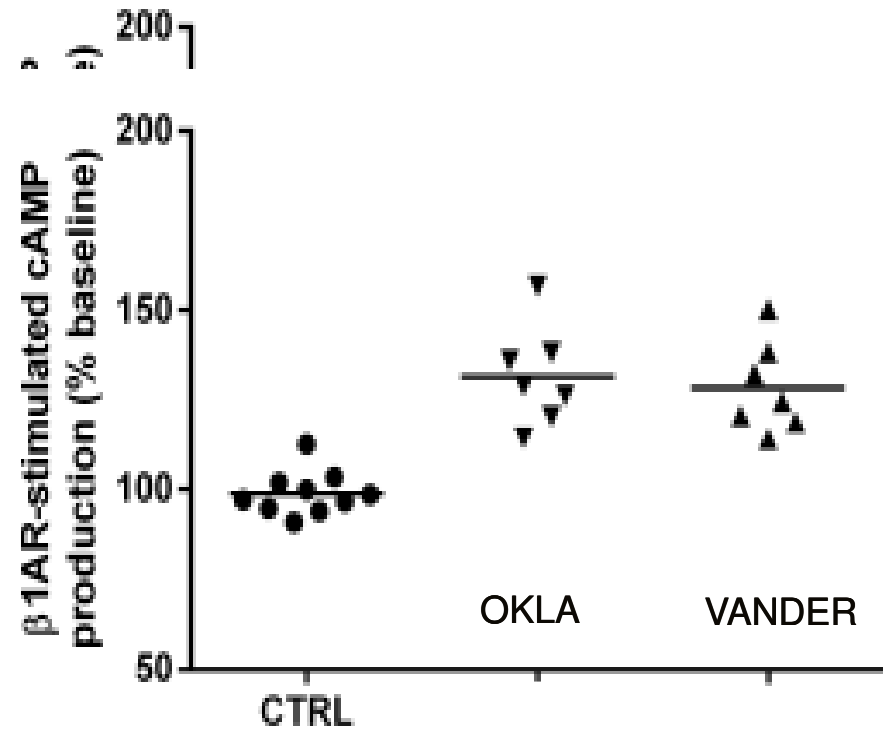
Results:

- Altogether, 15 of 18 ME/CFS patients with POTS or orthostatic intolerance (83%) had positive autoimmune findings to Beta-adrenergic receptors/PKA or M2 receptors ($P < .001$ vs. controls).
- Why was our positive autoimmune rate 83% vs. 30% for Loebel et al? Our ME/CFS patients all had symptoms of autonomic cardiovascular dysfunction (POTS or orthostatic intolerance), which is regulated by adrenergic and muscarinic receptors in heart, blood vessels and lymphatics.

- **#1. Autoimmune Basis for Postural Tachycardia Syndrome**
- Li, Cunningham, et al. JAMA 2014; 3: e000755
- Postural Tachycardia Syndrome (POTS) involves a rapid increase in heart rate when standing up, and is a symptom of autonomic cardiovascular dysregulation
- This investigation by the Oklahoma group looked at 14 patients with POTS and 10 healthy subjects. They examined autoantibodies to Beta-adrenergic and Alpha-adrenergic receptors for 2 reasons:
 - 1) they regulate HR and vasoconstriction vs. dilation, and
 - 2) nearly everyone has had Strep A which has a protein sequence is 85% similar to Beta-2 adrenergic receptors (molecular mimicry).
- These POTS patients did not have ME/CFS. But POTS or a related symptom (orthostatic intolerance) occurs in 25% of ME/CFS patients



Beta adrenergic receptor **gain of function** autoantibodies and Alpha adrenergic receptor **loss of function** autoantibodies are present in POTS patients. Increase in Beta 1 activity would increase heart rate in these patients. Increase in Beta 2 activity and/or decrease in Alpha 2 activity would decrease blood pressure (vasodilation).



B-Lymphocyte Depletion in Myalgic Encephalopathy/ Chronic Fatigue Syndrome. An Open-Label Phase II Study with Rituximab Maintenance Treatment.

Fluge Ø, et al. (2015) PLoS ONE 10(7): e0129898.

- Open-label study with 29 Norwegian ME/CFS patients.
- They were treated with Rituximab - two infusions two weeks apart, followed by maintenance Rituximab infusions after 3, 6, 10 and 15 months, and with follow-up for 36 months.
- Results showed lasting improvements in self-reported fatigue score in 18 out of 29 patients (62%).



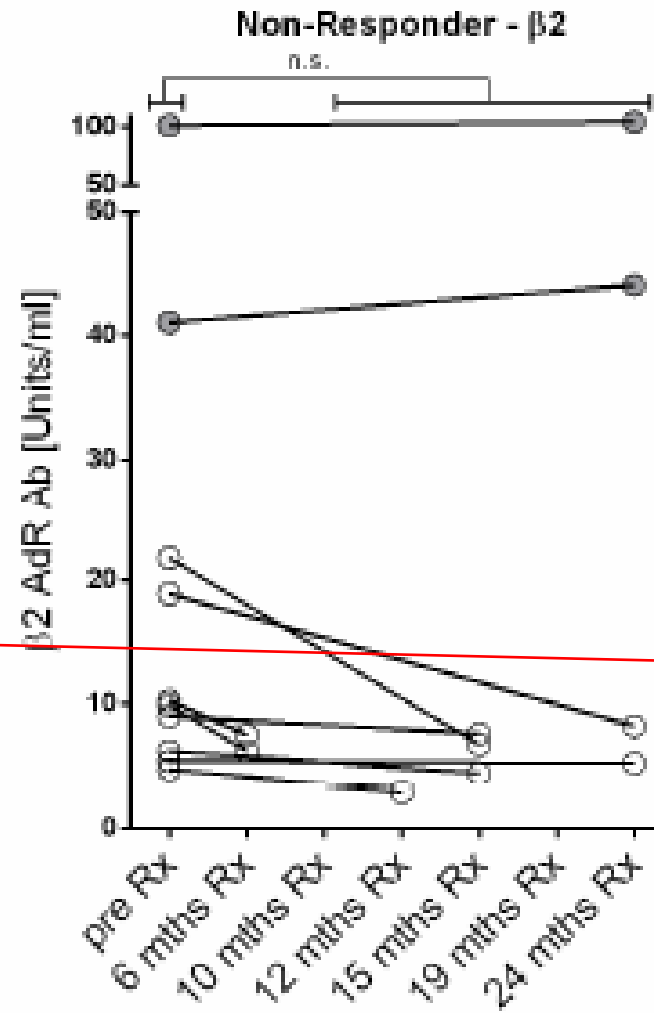
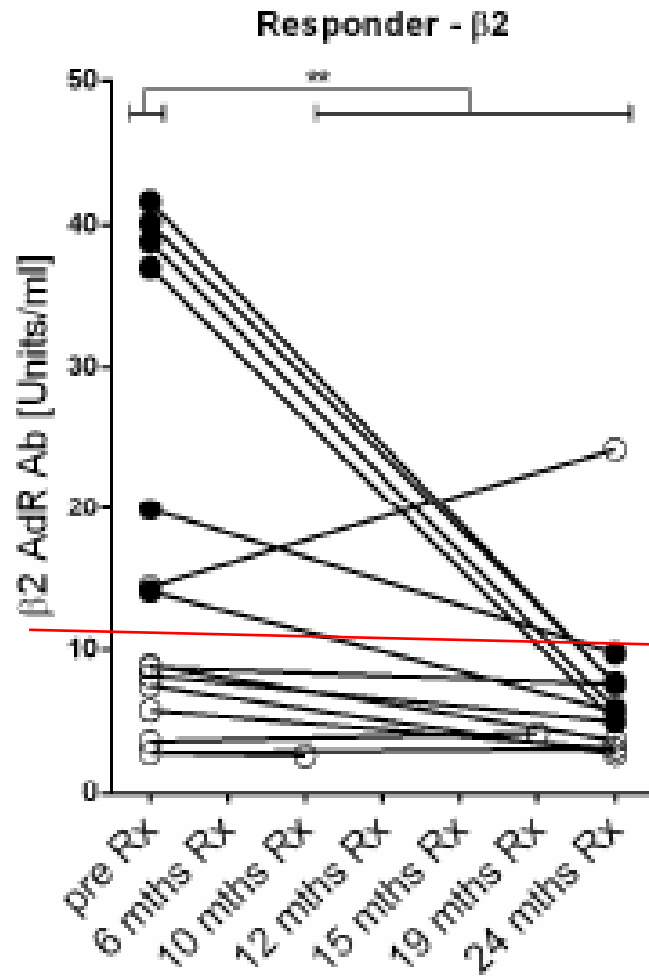
Antibodies to β adrenergic and muscarinic cholinergic receptors in patients with Chronic Fatigue Syndrome

Loebel et al *Brain, Behavior, and Immunity* (October 2015)

- This was a collaboration between a German group who are experts in Elisa for autoantibodies and the previous Norwegian group who did the Rituximab treatment trial. They tested serum samples for antibodies to multiple receptors in 268 ME/CFS patients vs. 108 controls.
- Approximately 30% of the ME/CFS patients had elevated antibodies to Beta-2 adrenergic or Muscarinic acetylcholine (M2-M5) receptors or both. (Both Beta-2 and M2-M5 receptors increase blood flow by vasodilation.)
- About 50% of the Responders from the previous Rituximab trial had high autoantibodies against Beta-2 adrenergic receptors before treatment. Most of them normalized after therapy. But other Responders to Rituximab had normal antibodies (below red line on next slide).



From Loebel et al 2015



Why have these variants not been found previously?

1. Heteroplasmy

2. Use of whole blood vs. WBCs

3. Many key genes are in areas of low reads

(see Figure)

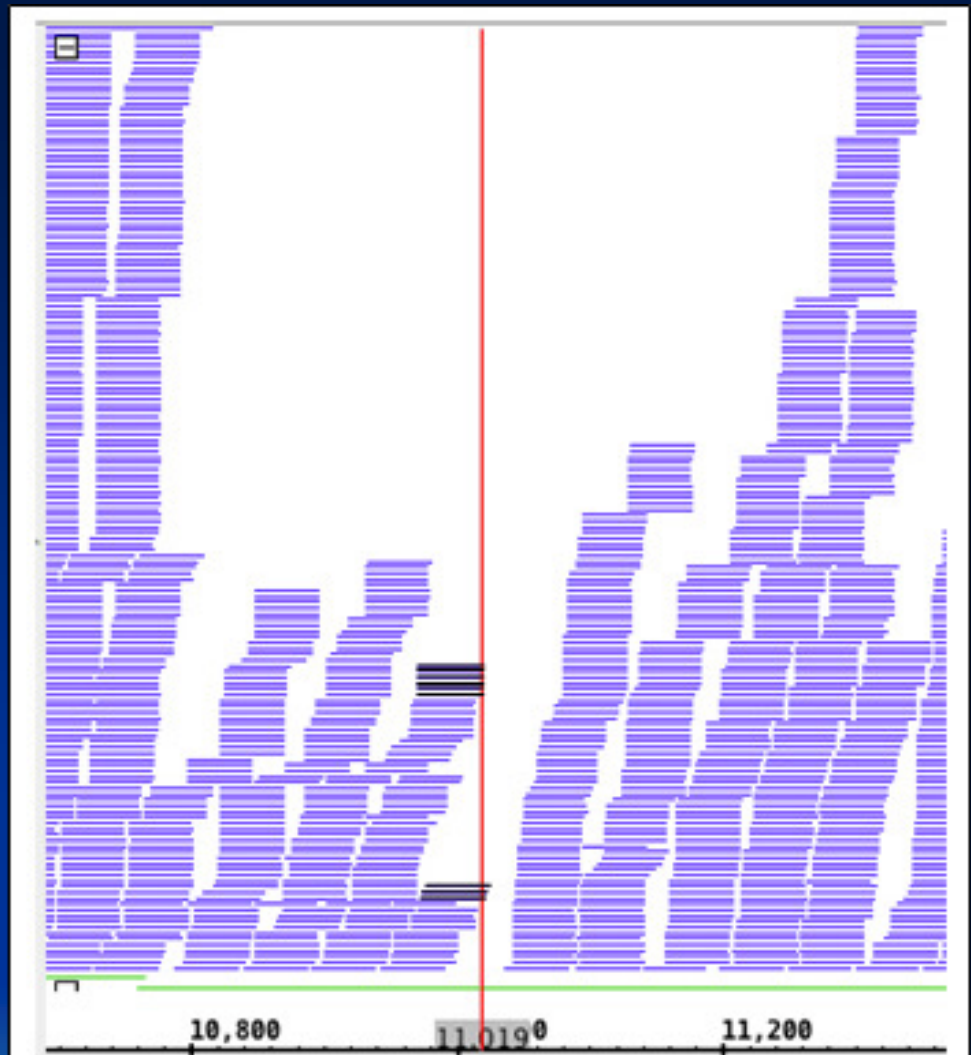


Figure 2. Graph of 50 base-pair reads near locus of high impact Mt-ND4 SNP (marked as red line) in one ME/CFS patient, showing valley of limited reads due to difficult sequencing region. All 9 reads at this locus (black horizontal lines) have the mutation (100% heteroplasmy). Other nearby reads shown in purple.

The causes of CFS+FM

Our hypothesis is that genetic susceptibility, autoimmunity and exposure to pathogens and stress all work together to cause CFS+FM:

1. Mitochondrial variants that affect ATP production in at least immune cells are necessary but not sufficient. These may be somatic or inherited. Alternatively or in combination, chromosomal variants affecting autoimmune regulation are necessary but not sufficient.
- 2. If these variants are present and together convey susceptibility to produce and sustain an autoimmune response, they may allow molecular mimicry that causes the production of autoantibodies that attack parts of the fatigue signaling system (e.g. Strep A that has a protein sequence that is 85% similar to beta 2 adrenergic receptors).
- 3. Physical or psychological stress may re-activate such autoimmunity, worsening symptoms.





HLA Mutation

Pathogen
(Epstein Bar, Strep-A, Flu,
Q-Fever)

T-Cells

B-Cells

Auto-antibodies

Fatigue System

Pain and Central Fatigue and Group III/IV muscle afferents,

7. Group III/IV signaling inhibits cortex neurons = Central Fatigue!

1. Motor Cortex Neurons

8. Group III/IV signaling Excites Anterior cingulate and other cortical neurons = muscle ache and pain ?

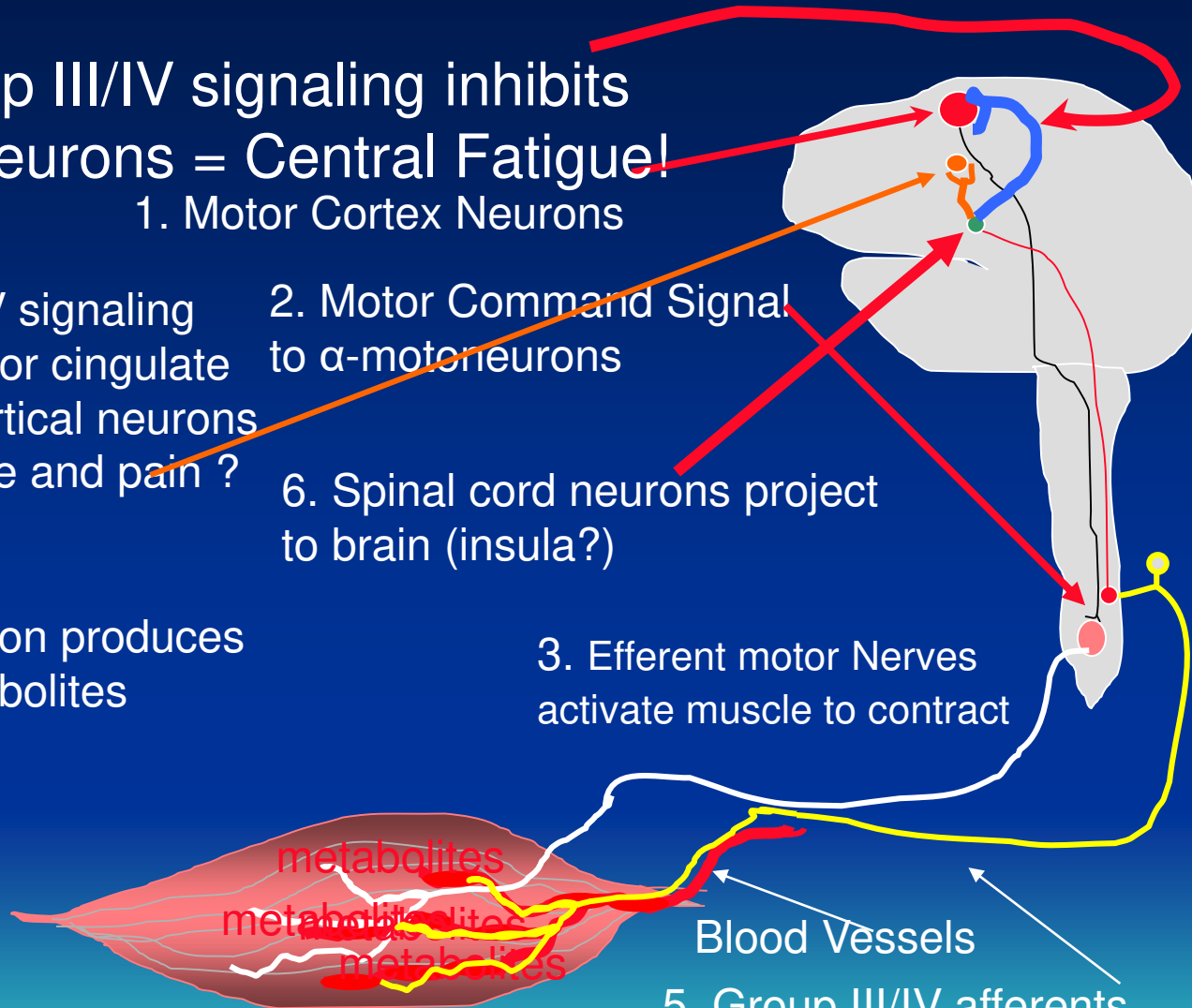
2. Motor Command Signal to α -motoneurons

6. Spinal cord neurons project to brain (insula?)

4. Contraction produces metabolites

3. Efferent motor Nerves activate muscle to contract

5. Group III/IV afferents on blood vessels detect metabolites project to spinal cord neurons



Group III/IV muscle afferents and Exercise Pressor Reflex

