Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) -a complex disease that has come of age with the SARS-CoV-2 pandemic

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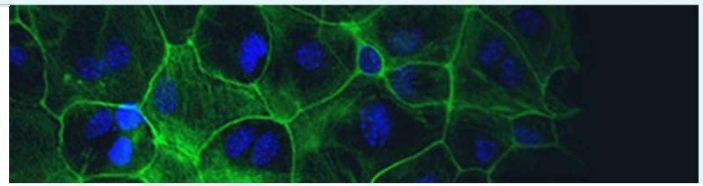
Department of Biochemistry
University of Otago,
Dunedin

Complex Chronic Illness Support
40th Anniversary Celebration
July 3rd 2021



What was my stimulus to become involved in ME/CFS research?

Began with the dramatic onset in 1990 of a mystery disease with pervasive fatigue in my young daughter

- Medical practitioners in Dunedin in 1990 knew nothing of the disease and the default view was it was likely a psychological 'perception' disorder

- despite NZ having an outbreak - 'Tapanui Flu' in 1984

- I put in a research proposal to the Health Research Council but reviewers questioned whether there was a real disease to study

The origins of ME/CFS

Documented Outbreaks of infectious diseases causing an ongoing post viral fatigue syndrome

Iceland 1946-49 (1090 cases) (**Akureyri disease**)

Royal Free Hospital London, 1955 (292 cases) (**ME**)

Incline Village Nevada 1984 (175 cases) (**CFS**)

A dominant global voice who firmly believed he had the answer to ME/CFS (1985-2005)

- - Sir Simon Wesseley (Professor of Psychological Medicine Kings College London
- **ME is "a perception disorder", an "opting out syndrome"**
- "a general disorder of perception, perhaps of both symptoms and disability. At the heart of this misperception lies the sense of effort. CFS patients clearly experience increased effort in everyday physical and mental tasks "

In NZ Medical, Social, and Societal attitudes towards ME/CFS

my 30 years experience

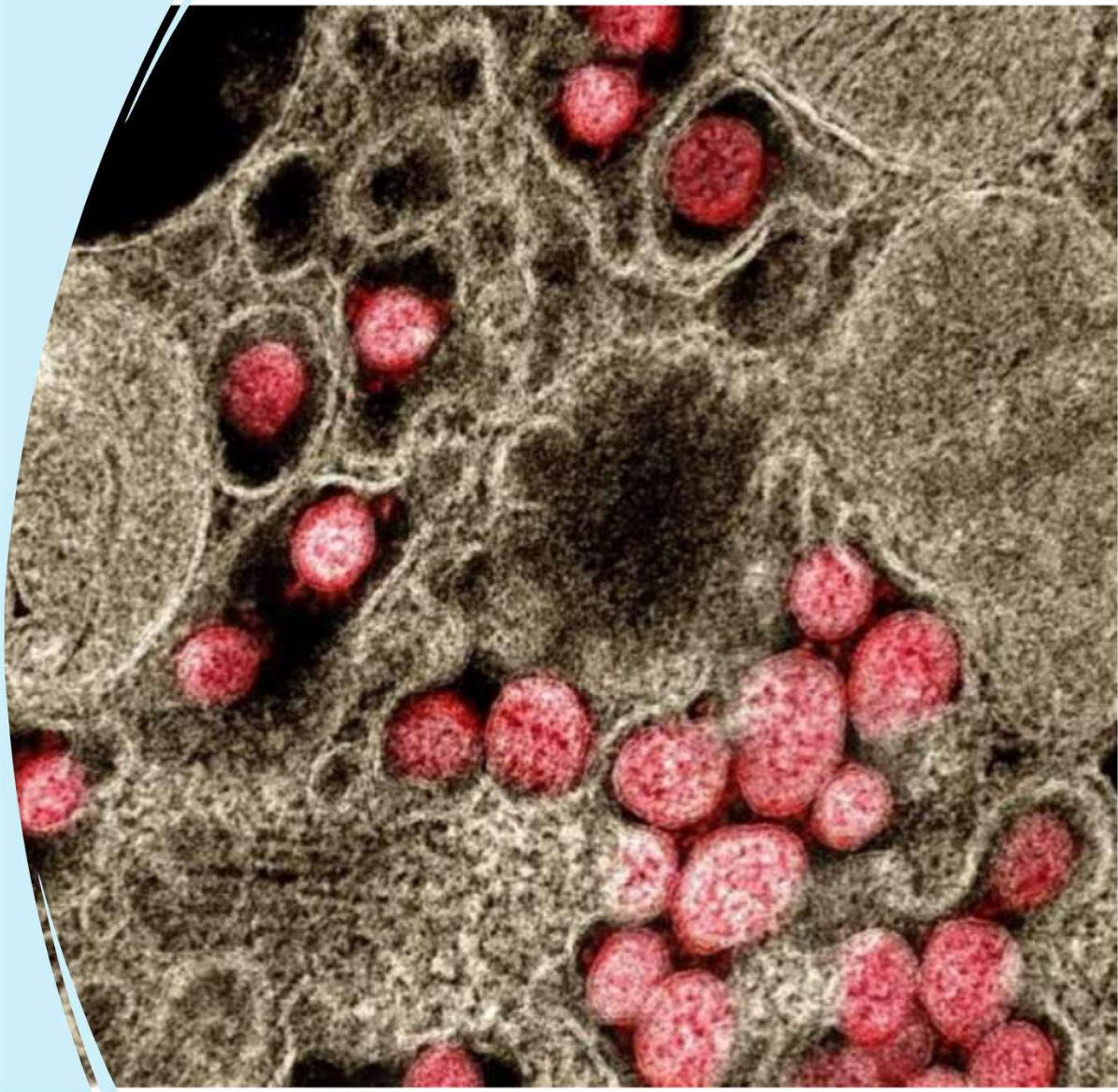
- Initially and even now among some a strong belief it is a somatic perception disorder
- Patients not having their illness affirmed by the health practitioners
- No medical school training and thereby knowledge
- Social services not accepting patients cannot work or need help
- Families not understanding –not a socially acceptable illness

Origins of Long Covid: arising from a specific viral infection

- SARS-CoV-2

(but now from 180 million infections! – it is not boutique!)

- a range of syndromes
  - Post viral fatigue
  - Ongoing lung pathology
  - Ongoing heart pathology
  - Post traumatic syndrome



Attitudes towards Long COVID?

- High degree of attention from the clinicians dealing with the pandemic - were surprised when patients did not recover as expected
- ME/CFS patients initially angry and now sympathetic but hope attention for their illness can coat tail.
- ‘Long haulers’ have had a strong voice through social media
- Special units set up in the UK to study ongoing the subgroups of ‘long hauler’ patients
- The NIH has promised over 1 billion dollars to support research into Long COVID
- In New Zealand – slow response from MoH given there are debilitated patients

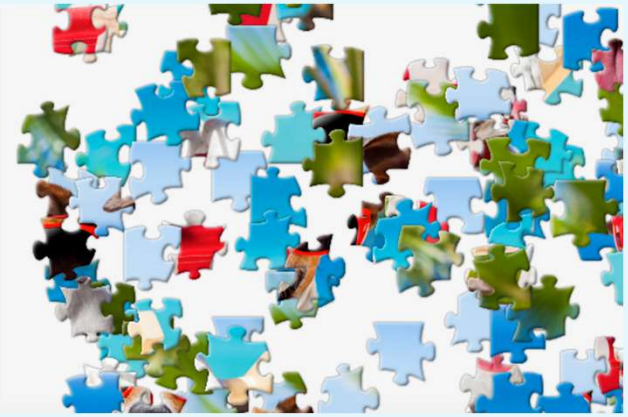
What is an ME/CFS patient like?

Changes in the USA in attitudes to ME/CFS in 2019 “just before the pandemic”

- The US senate passed a bill declaring ME/CFS was a serious illness
- Patients and caregivers had been poorly treated both medically and from social services
- Research support was promised from National Institute of Health – a few million dollars
- Research Centres on ME/CFS were set up at Harvard and Stanford

Solving the jigsaw puzzle of ME/CFS

2012



Lots of interesting individual pieces of research

No integrated picture of the illness

As a parent in an ME/CFS family my research questions were:

Onset: What '**control centre**' in the body is affected to cause such severe and diverse long-lasting symptoms?

Perpetuation: Why do the disease symptoms not resolve?

Relapse Cycles: What triggers the frequent relapses?

And then a new question was added on the 25th anniversary of my daughter's illness – aged 39

Physiological State: Why do the symptoms improve so markedly in **pregnancy –** at least for her?


SITY OF OTAGO
1869


Future
Otago
Graduate

Now a pre-teen on his 6th birthday

How do we get started on research with modest resources? Around 2012!

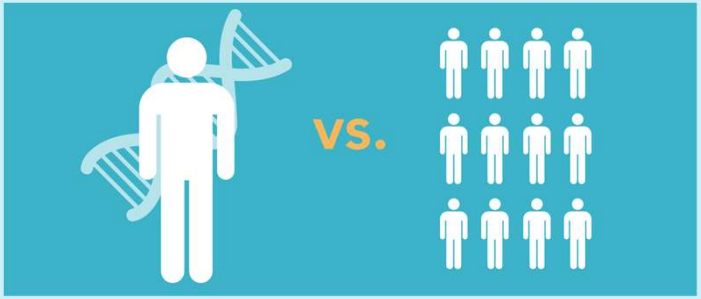
- **Approach**:

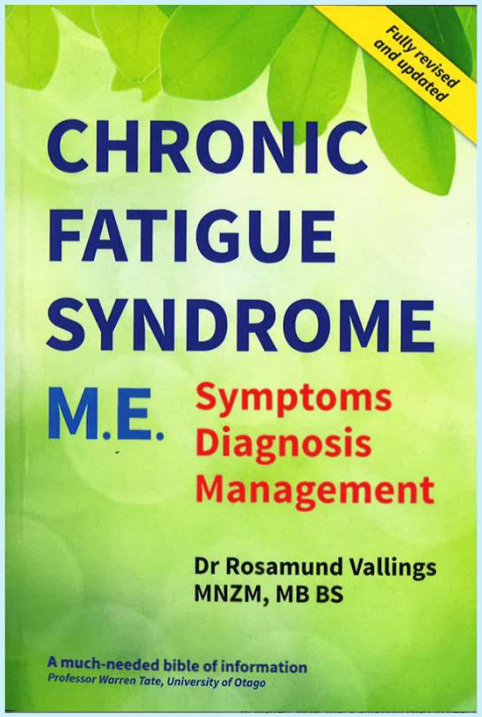
Inspired by 'Personal omics study' of Michael Snyder over 2 years of Snyder's own personal health – multi$M -40 authors 2012 Cell paper

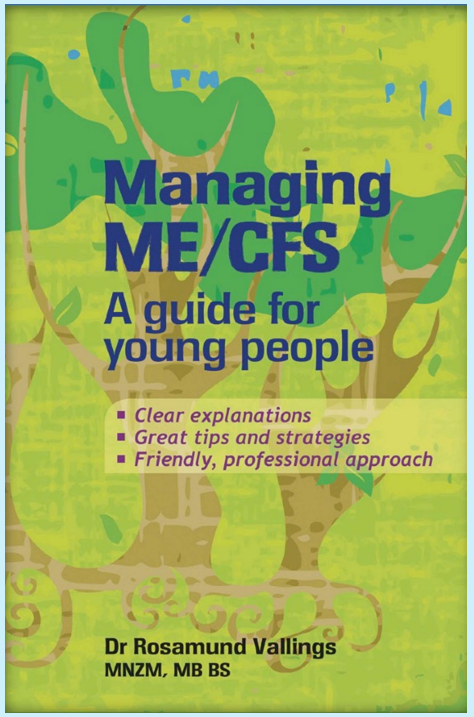
- **Size of patient studies**

Inspired by my former student, Tony Manning, Momenta Pharmaceuticals -study of rare diseases with very few patients

- **Used precision medicine approach**

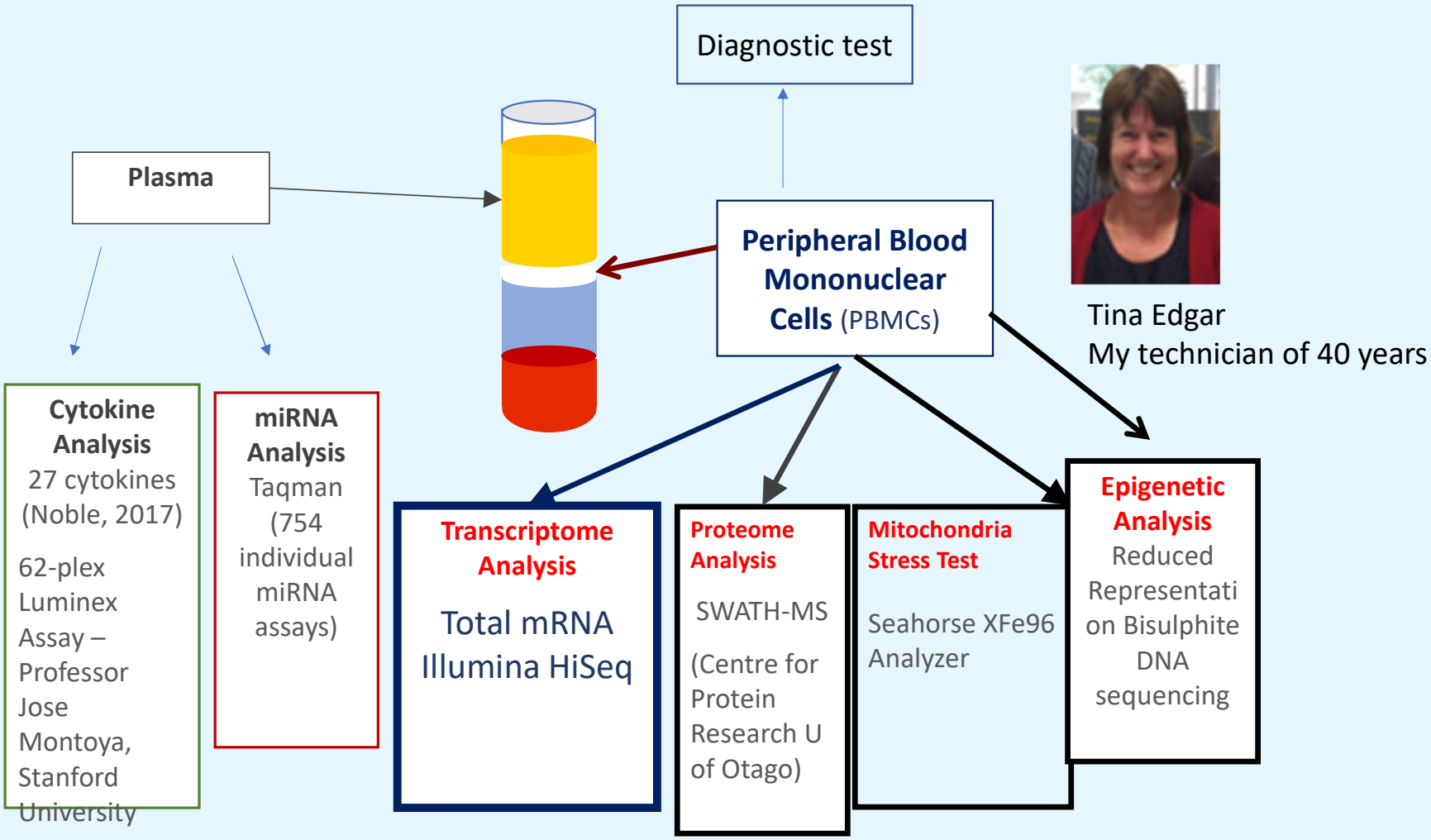






NZ ‘s expert GP for ME/CFS: Ros Vallings

Our molecular studies with ME/CFS patients

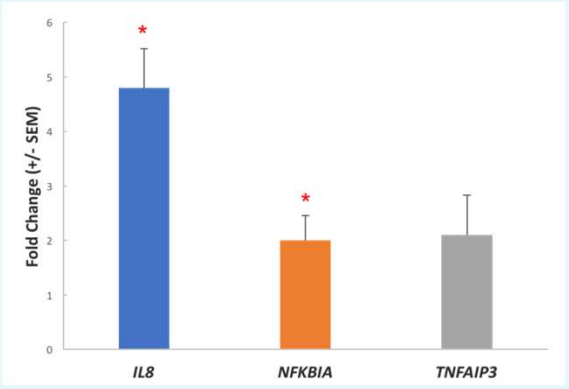


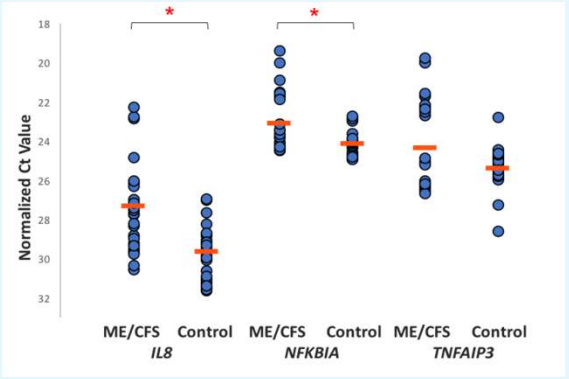
Active genes in immune cells -13000 transcripts

Top 3 changes in ME/CFS involved in inflammation

- 27 gene transcripts increased and 6 decreased (P<0.01).

Gene	Name	*P*-value	Fold Change
IL8	Interleukin-8	2.5×10^{-6}	5.6
NFKBIA	NF-κB Inhibitor Alpha*	2.0×10^{-5}	2.4
TNFAIP3	Tumour Necrosis Factor Alpha-Induced Protein 3*	1.3×10^{-4}	3.6





Eiren Sweetman

- Anti-inflammatory responders to TNF-induced NF-κB activation

As a PhD student

Functional Pathways

- Functional Network Analysis (n=33, $P<0.01$) and Ingenuity Pathway Analysis (n=165, $P<0.05$) detected effects on:
  - **Mitochondrial function (energy production)**
  - **Cellular stress response/oxidative stress**
  - **Lowered Metabolism**
  - **Immune/inflammatory pathways**
  - **Circadian clock function**

Sweetman, E., Ryan, M., Edgar, C., MacKay, A., Vallings, R., & Tate, W. (2019). Changes in the transcriptome of circulating immune cells of a New Zealand cohort with myalgic encephalomyelitis/chronic fatigue syndrome. *International Journal of Immunopathology & Pharmacology*, *33*, 1-8

Cellular proteins – immune cell proteome

Mass spectrometry Analysis

Eiren Sweetman

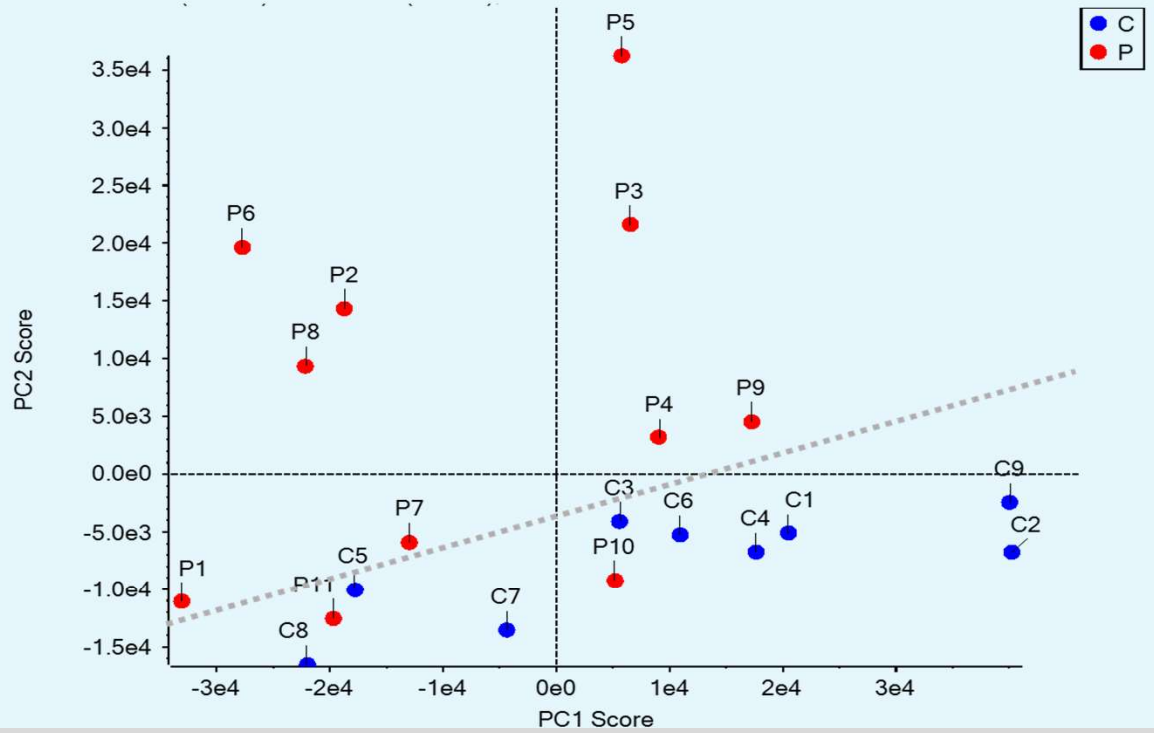
1. **Digested isolated proteins into tiny pieces**

2. **Built a spectral library with small samples from each of all the patients and controls (~3000 proteins)**

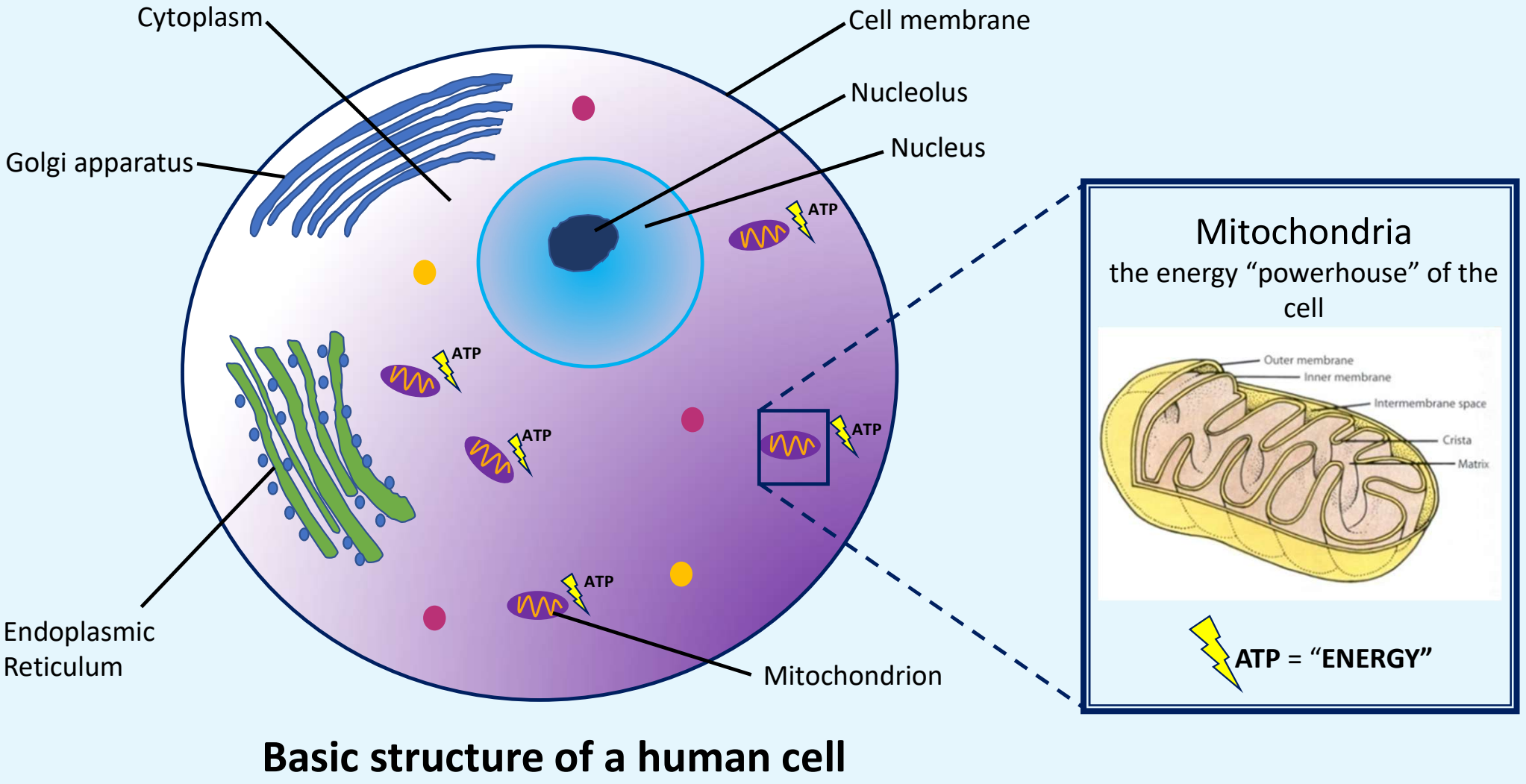
3. **Quantify proteins in all samples :Sequential Acquisition of all theoretical Fragment Ion Spectra mass Spectrometry (SWATH-MS)**

Principal Component Analysis (PCA)

A principal component analysis (PCA) was used to stratify groups based on protein abundance patterns, which segregated the majority of the ME/CFS patients from the controls.

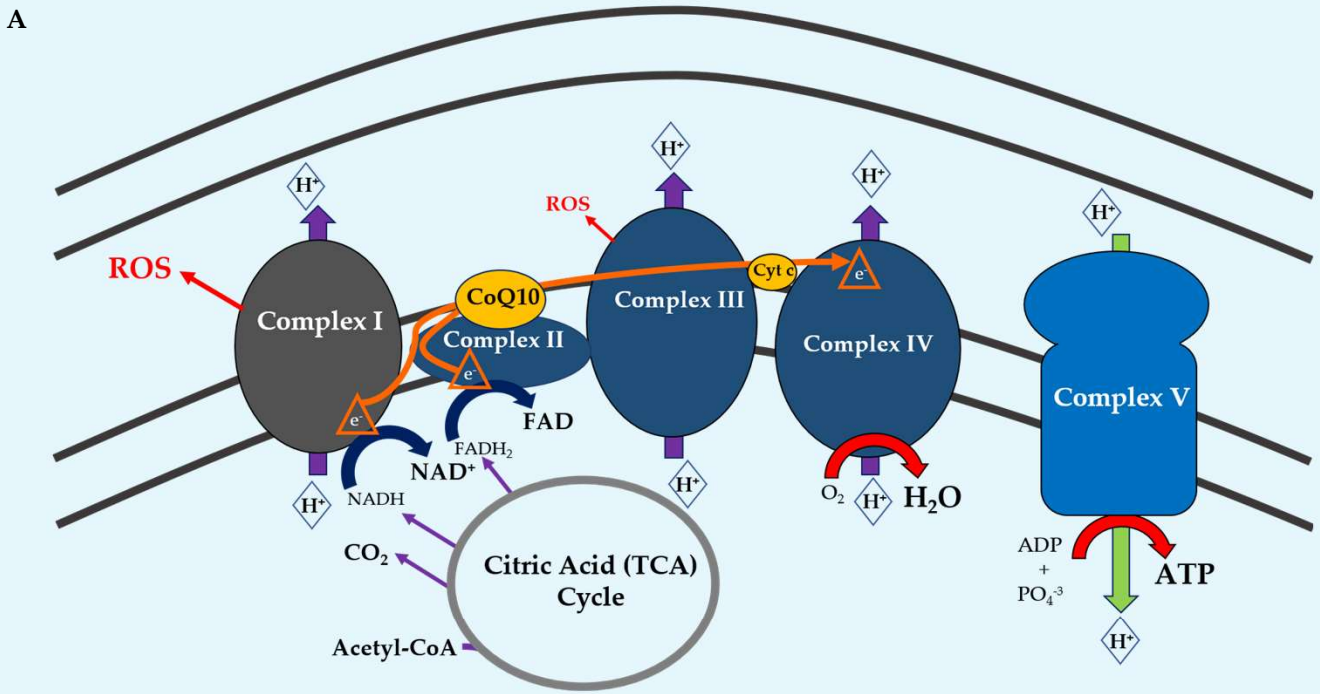


9/11 ME/CFS patients separated from healthy controls (linear regression analysis)



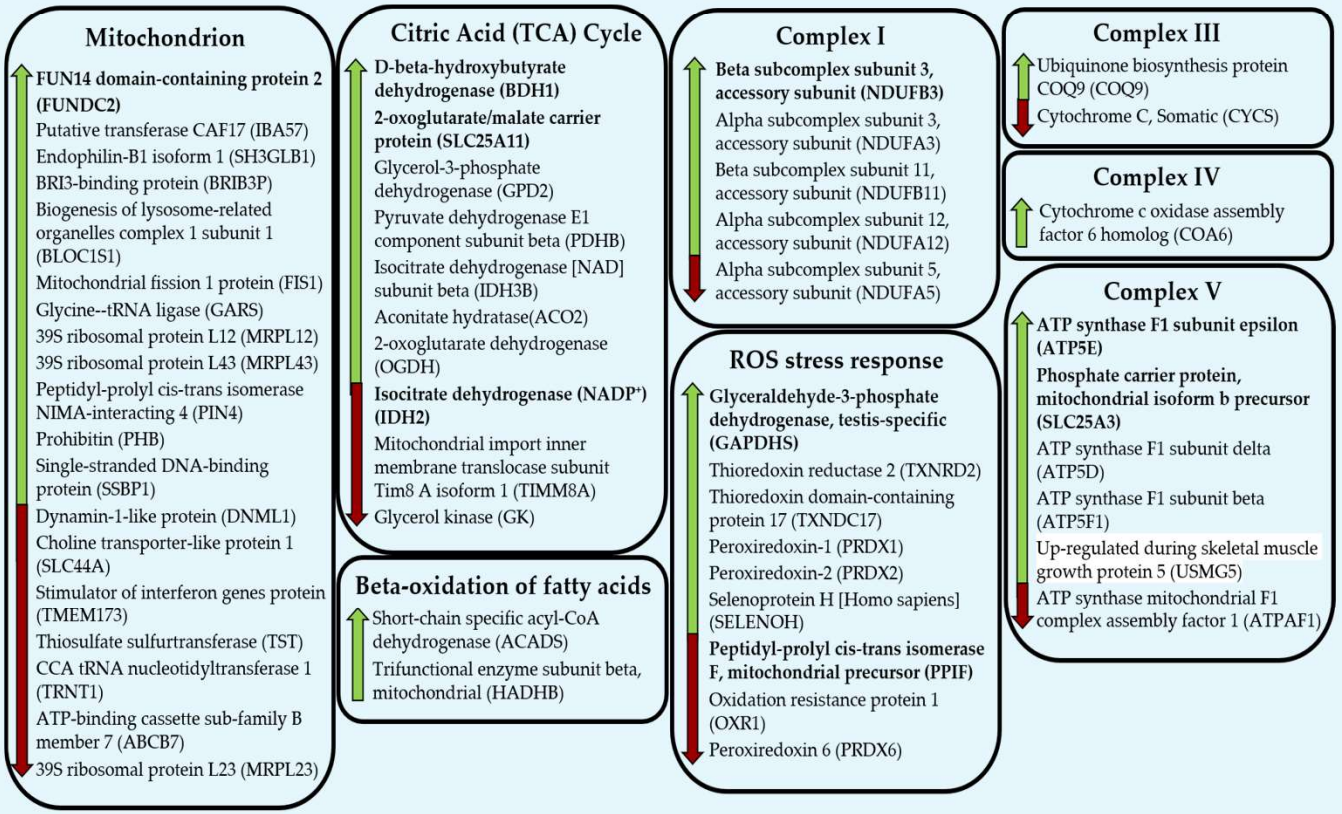
Basic structure of a human cell

Key finding: Many mitochondrial proteins up or down regulated



Sweetman ES, Kleffmann T, Edgar CD, DeLange, M,, Vallings R, &Tate WP (2020) A Swath-MS analysis of Myalgic Encephalomyelitis /Chronic Fatigue Syndrome peripheral blood mononuclear cell proteomes reveals mitochondrial dysfunction *J Translat Med 18:365*

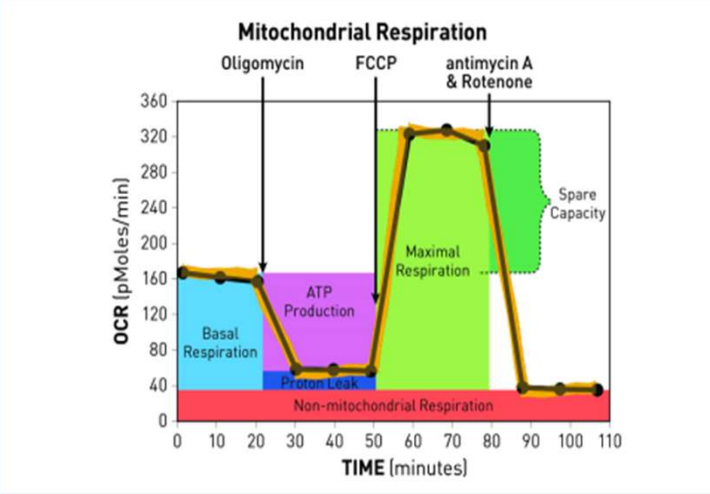
Mitochondrial proteins affected –structural, metabolic pathways, electron chain complexes , ROS response



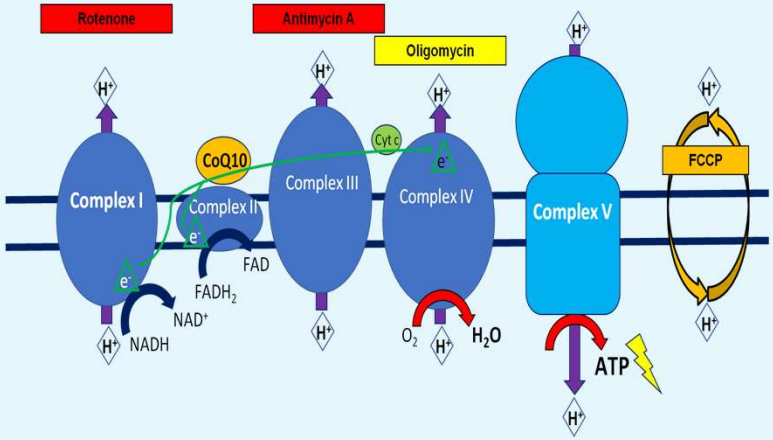
Seahorse analysis of Mitochondria

Mito Stress Test

The test measures mitochondrial function by directly measuring the oxygen consumption rate (OCR) of live cells to deduce

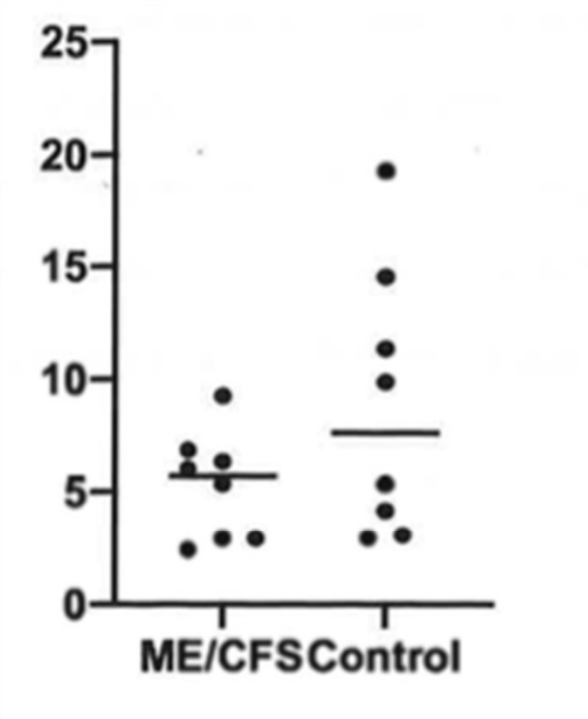


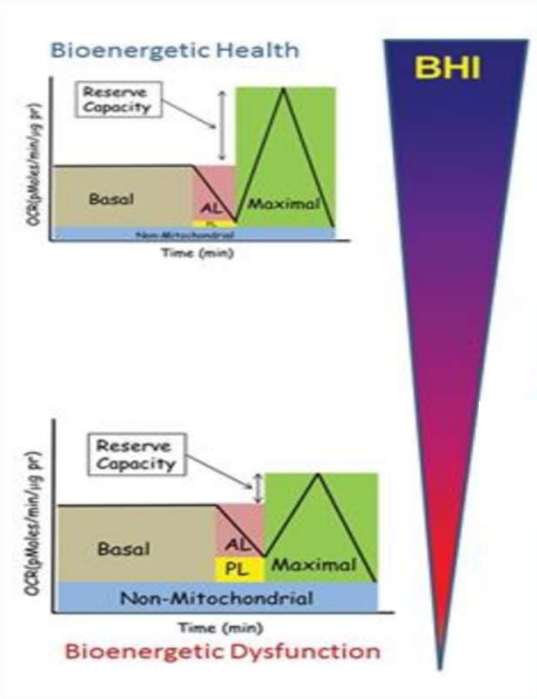
ATP production , Maximal respiration, Non-mitochondrial respiration, Proton leak and spare respiratory capacity

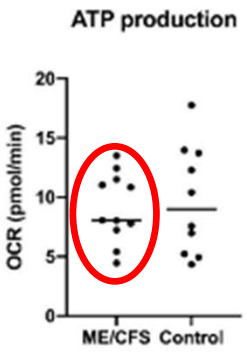


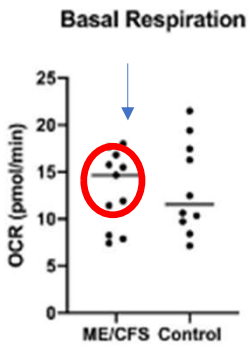
Bioenergetic Health Index

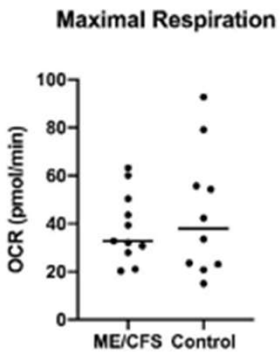
Results suggest a lower BHI in ME/CFS

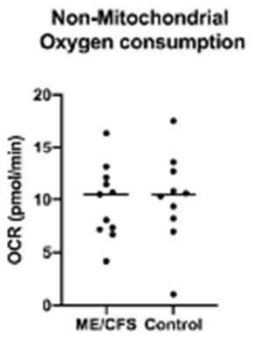


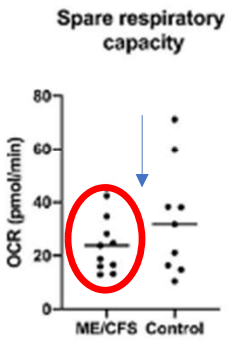


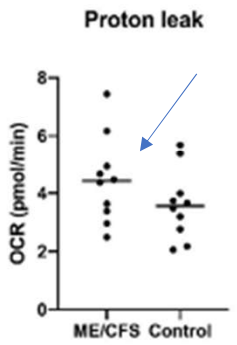




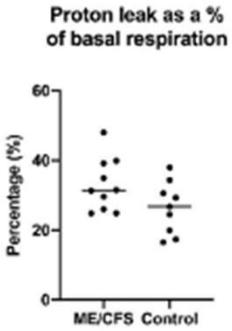








Tina Edgar



Emily Wood
Med 3 research year

My big questions as a parent in an ME/CFS family

Control centre for the disease?

symptoms, non recovery, relapses

brain

Why not recovery?

Ongoing stressor

Explanation

neuroinflammation?

A neuroinflammatory paradigm to explain symptoms, lack of recovery, and relapses in ME/CFS

Angus Mackay

Focused on the hypothalamus and the 'stress centre of the brain' – a cluster of neurons within the paraventricular nucleus

Paradigm: "Cycles of fluctuating chronic neuro-inflammation can drive stress related relapses in ME/CFS and support the failure to recover the 'normal' homeostatic set point that would allow recovery"

We think damaged mitochondria have a role as the danger signal

Mackay, A & Tate WP (2018) A compromised paraventricular nucleus within a dysfunctional hypothalamus: A novel neuroinflammatory paradigm for ME/CFS *Internat J Immunopath and Pharmacol* 2: 1–8

Changes in epigenetic code of the DNA associated with ME/CFS

Changes during relapse recovery cycle

Amber Helliwell

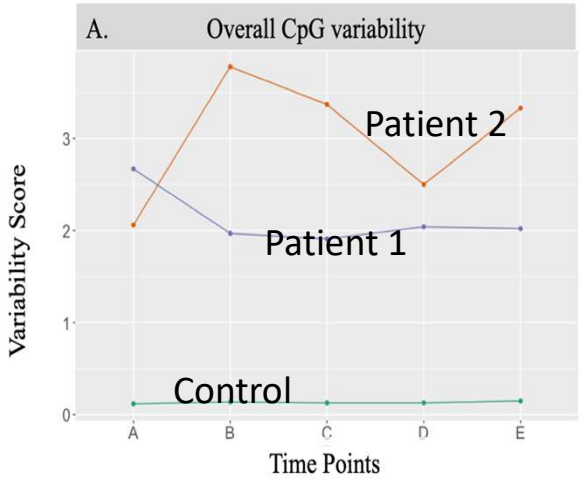
Aniruddha Chatterjee (Pathology)

A. Summary of self reported health scores (A-E)

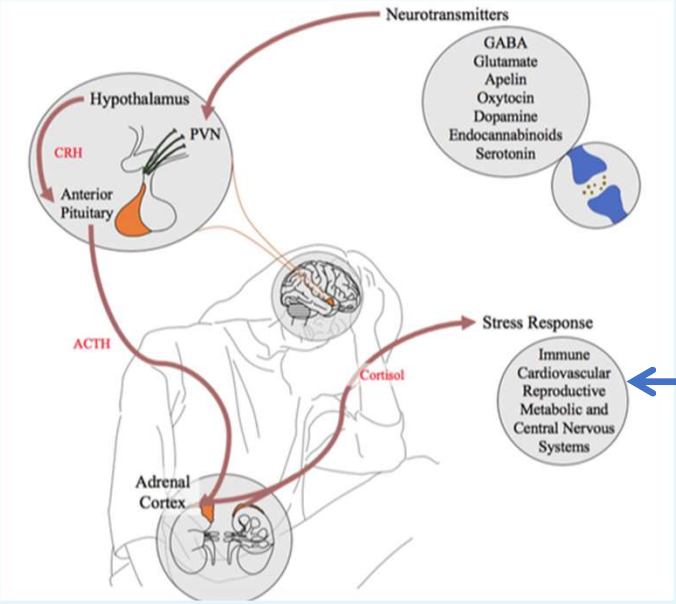
	A	B	C	D	E
Patient One	"Well" 7	"Fragile" -3	"Fragile" -2	"Satisfactory" 7	"Satisfactory" 7
Patient Two	"Fragile" 5	"Fragile" 6	"Relapsed" 2	"Satisfactory" 6	"Fragile" 4
Control	"Well" 10	"Well" 10	"Well" 10	"Well" 10	"Well" 10

Generated reduced genome DNA methylation maps
By reduced representation bisulphite sequencing

Patient epigenetic methylome code was
20 fold more dynamic than the control



Hypothalamus/Pituitary/adrenal (HPA) axis with neurotransmitter pathways identified as affected in ME/CFS

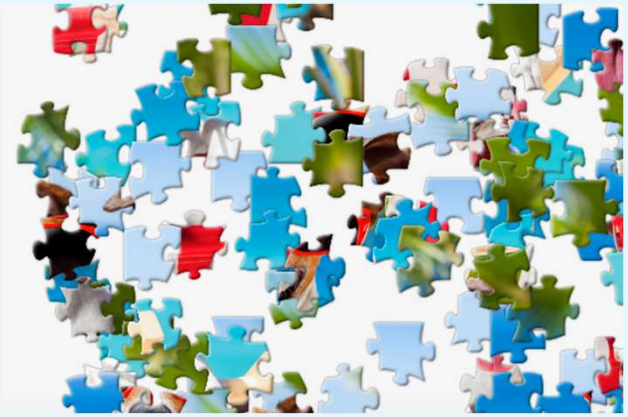


Effects on neurotransmitters identified by five hypomethylated gene regions:
RYR1, GNAS, GNG7, GABRB3 and APBA2

Low cortisol associated with **fatigue and Post Exertional Malaise**

Solving the jigsaw puzzle of ME/CFS

2021



Lots of interesting individual pieces of research

An integrated picture of the disturbed physiology
In ME/CFS

What now in 2021 & 2022?

A. The most extensive study yet of **Post Exertional Malaise** – a core symptom of ME/CFS
(i) Cardiac Physiology (ii) DNA methylome (iii) mitochondrial function
(iv) Oxidative stress biomarkers in Protein and DNA, glutathione oxidation status

Jemma Elley

Tina Edgar

Anna Blair

B. Long COVID – a comparative study with ME/CFS
does it have the same molecular signatures?
(i) Immune cell proteome (ii) DNA methylome
(iii) Oxidative stress

Planned

C. Long COVID immune cellular and molecular signatures
before and *after* vaccination (with Anna Brooks - specialist immunologist UoA)
(i) Serology (ii) Specific immune cell dysfunctions (iii) molecular signatures

Dr Anna Brooks

Acknowledgements

Collaborators

Rosamund Vallings –Howick Health and Medical Centre
Torsten Kleffmann – Protein Chemistry Centre
Aniruddha Chatterjee – Dept of Pathology
Michael de Lange – Biostatistics
Margaret Ryan – Dept of Anatomy
Peter Stockwell – Dept of Pathology
Lynette Hodges , School of health and exercise Massey U
Anna Brooks – School of Biological Sciences UoA

Collegial Discussion

Paul Fischer – Monash U, Vic
Jose Montoya – Stanford, Calif
Paul Naviaux – Stanford, Calif
Sonya Marshall-Gradnisek Griffiths U, Que

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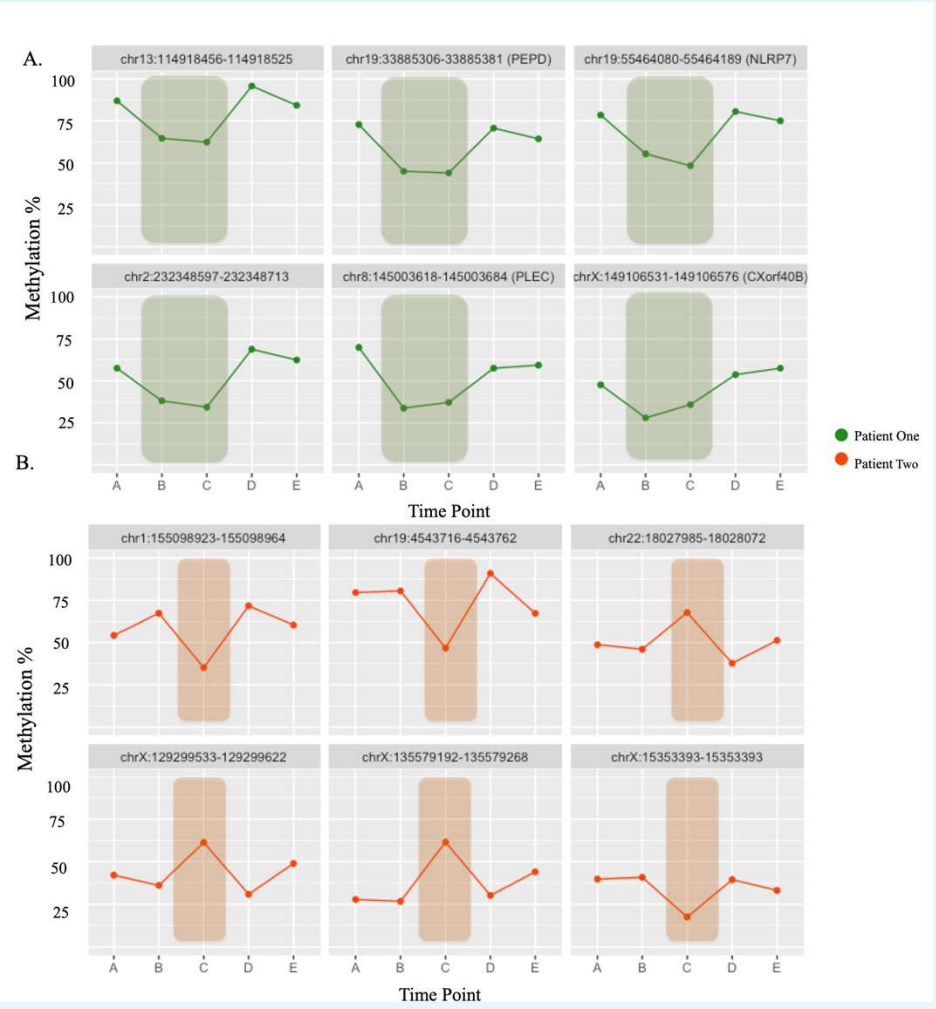
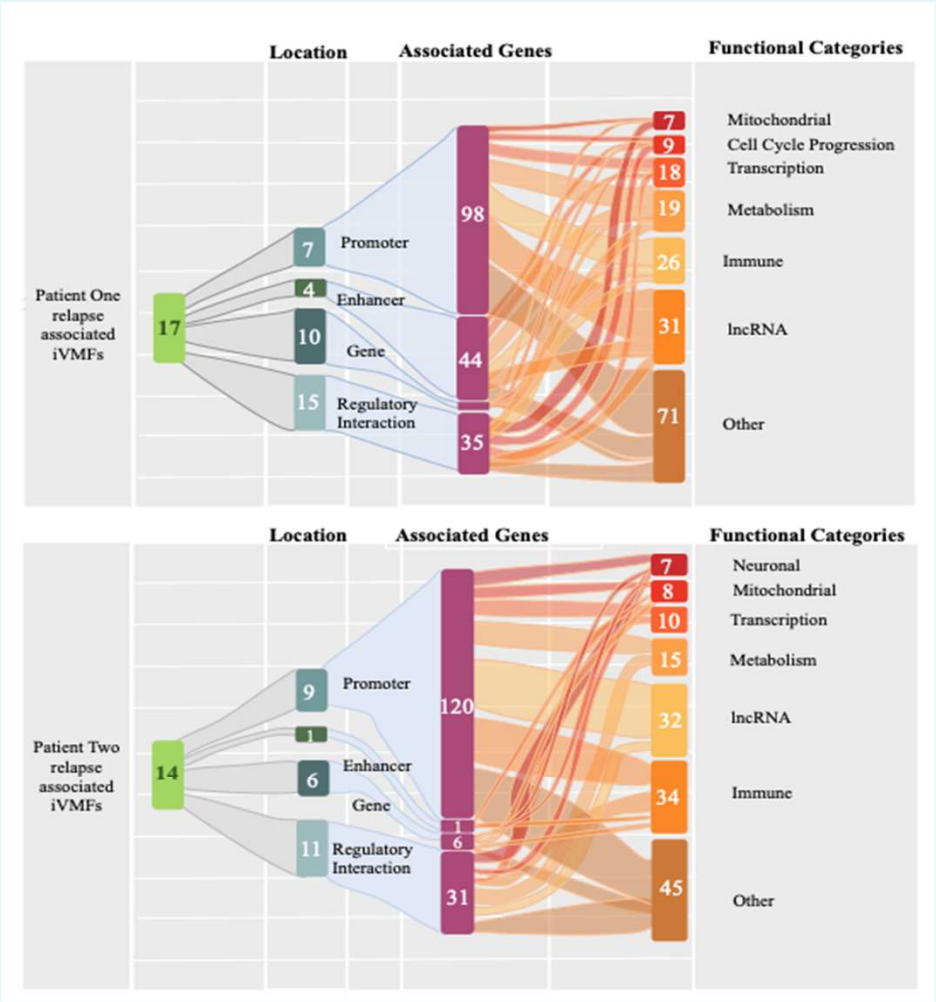
We also wish to express our sincere gratitude towards all of our ME/CFS study participants.

DNA methylation changes at specific sites



Summary of relapse associated features and functions



First Study Group

Clinical characteristics	ME/CFS participants	Control participants
Number	11	9
Median age (years)	43.2	38.0
Age range (years)	11.3-69	12.5-60
Gender	F=7	F=6
	M=4	M=3
Median BMI	23.7	[b]
Nationality	NZ/European	NZ/European
Median illness duration (years)	11	N/A
Stage of illness[a]	Acute=2 Chronic=9	N/A
Potential initial ME/CFS 'trigger'	Acute infection=7 Surgery=1 Stress=1 Unsure=2	N/A

[a] self-reported

[b] Data not collected

All of our ME/CFS participants were diagnosed by Dr Vallings using the 2003 Canadian Consensus Criteria