

**Report of the third UK CFS/ME Research
Collaborative conference
Newcastle
28-29 September 2016**



Introduction

More than 90 scientists and people affected by CFS/ME came together at the third annual UK CFS/ME Research Collaborative (CMRC) conference in October.

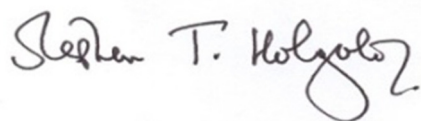
Travelling from countries including Canada, Australia and North America, delegates heard about the exciting launch of the new ME/CFS Epidemiology and Genomics Alliance project (MEGA) and watched presentations exploring a range of topics including big data, biomarkers, pain and autonomic dysfunction.

It was immensely encouraging to see new faces, and to hear the enthusiasm and commitment from not just those with experience in the field of CFS/ME but also those bringing in expertise from other disciplines. On behalf of the Executive Board of the CMRC Executive Board I extend a sincere thank you to all those whose energy and enthusiasm made the event possible.

I would also like to thank the following specifically:

- Karen Hainsworth, for contributing to this report
- Katrina Pears, Volunteer, Action for M.E., for contributing to this report
- Emily Beardall, Volunteer Pharmacist, Action for M.E., for contributing to this report
- Sonya Chowdhury, Chief Executive, Action for M.E.
- Clare Ogden, Head of Communications and Policy, Action for M.E.
- Joe Martin, Communications Officer, Action for M.E., who filmed the conference presentations for livestream: you can view these online at www.tinyurl.com/actionformeyoutube

As we move forward with MEGA – you can read more about this throughout the report – I hope that you share my excitement and optimism about changing the field of CFS/ME research. Thank you to all those who continue to support the work of the CMRC, and who push for the collaborations needed to effect real progress.



Stephen Holgate
CMRC Chair

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To find out more about the CMRC, including how to become an Associate, Student or Professional Member, please visit www.actionforme.org.uk/CMRC

Welcome

Prof Stephen Holgate, UK CFS/ME Research Collaborative Chair

“This is the third conference at a time of terrific excitement in the field [of CFS/ME],” said Prof Holgate in his welcome address. “It’s exciting because the world is beginning to wake up now to new ways of approaching this complex group of conditions.”

He was referring specifically to -omics, the mapping and analysis of large scale biological information. Fundamentally different from standard research, -omics produces huge volumes of data allowing researchers to unpick disease sub-types and come up with new ways of diagnosing and treating them.

Prof Holgate hailed two recent studies from the US that showed the true potential of this approach. In the paper *Metabolic features of chronic fatigue syndrome*, Naviaux et al looked at differences of over 600 plasma metabolites from 63 biochemical pathways in CFS/ME patients compared to controls: “What was quite remarkable, when they did their analysis, [was that] they got a very distinct collection of metabolites that emerged in these relatively severe CFS/ME patients.”

Prof Holgate noted that there were several differences between males and females too. In a nutshell, CFS/ME appeared to be a reduced metabolic (hypometabolic) state caused by a variety of environmental insults, and this state seemed to trace back to the cells powerhouse – the mitochondria.

Flagging up a different and smaller research project by Hanson et al, he was excited by the correlation in results. “I think this is a real breakthrough. What’s interesting about these two studies is that despite the fact they were using different methods and different analytical procedures they came up with a very similar answer.” An attempt is already being made to repeat this research.

Scientists in the UK are also keen to use metabolomics to investigate CFS/ME, said Prof Holgate, and highlighted the work of the ME/CFS Epidemiology and Genomics Alliance (MEGA).

Led by Prof George Davey-Smith, a clinical epidemiologist in the Medical Research Council (MRC) Integrative Epidemiology Unit, Bristol, MEGA contains expert scientists from across the UK. One of the collaborators is Dr Warwick Dunn, from the new Birmingham Phenome Centre, who has promised that CFS/ME will be a key focus in his lab.

At the time of the conference, Dr Dunn was at a metabolomics meeting at the National Institute of Health (NIH), the main research funding body in the US. He did however send a message of support, saying that Prof Francis Collins, head of the NIH, had highlighted the Hanson metabolic paper as a real achievement. “So CFS/ME is important to important people,” he concluded.

Prof Holgate agreed, saying, “If somebody like Collins is saying this is a great example of metabolomics, then that should give us great confidence.” He added that efforts will be made to link to -omics activities in this disease area in the US and beyond.

Prof Holgate then laid out how MEGA would proceed. “Starting with genomics and DNA sequencing but then spreading all the way through the cellular processes, through transcriptomics, proteomics, metabolomics and even microbiomics and serumomics, we’ll be looking to pull this all together and come up with some pathways.”

There was one important element he was keen to stress. “We can have all the -omics in the world but unless we get the patients and the phenotyping right then it’s all a waste of time.”

After thanking the conference organisers and delegates, Prof Holgate expressed the deep need for continued commitment to and enthusiasm for this project from scientists, patients and the CFS/ME community. This, he said, was essential to get the study funded. Presaging the new and exciting era to come, he concluded, “We are on the threshold of an extraordinary journey.”

BIG DATA, BIOMARKERS AND STRATIFICATION

UBC Complex Chronic Disease Study: How do you research a syndrome?

Prof David Patrick, University of British Columbia

Key point summary

- The main study looked at four groups - CFS/ME, alternately-diagnosed Lyme syndrome (ADCLS), lupus and healthy controls – using metagenomics, transcriptomics and immunosignature chips
- Patients who had been diagnosed with ADCLS were negative for Lyme and similar to those with CFS/ME
- CFS/ME patients had a disability score precluding them from work, and these were mild-moderate patients, so people with severe CFS/ME are more disabled
- There was no distinction between the illness groups and healthy control group on mental health scores, so this would not support an argument that mental health was a causal factor for CFS/ME
- In a sub-study of sub-maximal exercise testing, CFS/ME patients were unable to exert the same amount of force as the healthy controls but the procedure did not distinguish clearly enough between CFS/ME cases and healthy controls for it to be used as a diagnostic test
- The metagenomics paper currently in review reports that there were no large differences between the viral and bacterial content of blood between people with CFS/ME and healthy controls
- There is some potential to define a specific disease signature using immunosignature work
- Clinical trials and prospective cohort studies, or long term population based studies, are needed in order to establish causality in CFS/ME.

Prof Patrick is an infectious disease epidemiologist who has studied HIV, SARS and pandemic flu. He began by introducing the large collaborative he works with on the University of British Columbia's Complex Chronic Disease (UBC CCD) study which spanned several research specialisms including biochemistry, physiology, rheumatology, microbiology, immunology and epidemiology, along with clinicians. The study has had input from transcriptomics, CPET and immunosignature assay experts, as well as collaboration from groups at UCSF, Arizona State University, Workwell Foundation and University of Bergen.

Aetiology of CFS/ME

There is a history of diseases with unknown cause turning out to be infectious diseases. Stomach ulcers were thought to be caused by stress and lifestyle factors but it was eventually discovered that they are caused by *H. Pylori* bacteria. Other examples include tuberculosis, cervical cancer, and possibly asthma (where *missing* microbiota may play a role) – so why not CFS/ME?

CFS/ME has an unknown aetiology, with a high level of disability compared with other chronic diseases. 1.4% of Canadians report a diagnosis, though fewer would fit a research

definition. Putative aetiologies from the past include viruses (eg. EBV, HHV-6, HHV-7, XMRV), bacteria (Borrelia, Chlamydia), environmental triggers, mitochondrial dysfunction, and the hit and run hypothesis. The latter states that a stressor, such as life stress or an infection, sets the body in chronic disequilibrium.

Rather than picking individual potential causes and then studying them, -omics investigates across the board without a hypothesis, so this is more likely to give us new clues to the cause of CFS/ME, said Prof Patrick.

In CFS/ME, the pathophysiology is unknown and there are no reliable biomarkers, which leads to imperfect case definitions and misclassification. Researchers are likely to be studying a mixture of different disorders, which makes it more difficult to find key differences. Prof Patrick welcomed efforts to study much larger sample sizes, which will make these different disorders more apparent.

Reviewing recent studies, he noted that Hornig and Lipkin (*Molecular Psychiatry*, Feb 2016) discovered a disturbed cytokine signature in the cerebrospinal fluid of people with CFS/ME. Naviaux and team (*Proceedings of the National Academy of Sciences of the United States of America*, Sep 2016) found metabolic differences between CFS/ME and healthy controls. Fluge and Mella (*PLoS One*, Jul 2015) have had success in their B cell depletion trials with rituximab.

But Prof Patrick urged caution with the cytokine and metabolism findings, as there has been false hope before, with XMRV. Clinical trials and prospective cohort studies, or long term population based studies, will help establish causality by studying healthy people entering the studies and turning out to have CFS/ME.

UBC CCD study

Although the UBC CCD study is a small one, its aim was to look at CFS/ME in new ways, and make observations that lead to a hypothesis for new study designs. The platforms employed are:

- metagenomics – high throughput sequencing for viral and bacterial discovery
- transcriptomics – the same sequencing technology but focusing on mRNA in host gene expression, as a way of telling which genes are switched on and which are switched off
- immunosignature chip – to interrogate the antibody repertoire to look at the infection history and autoimmunity

The study enrolled people with the Canadian 2003 definition of the illness and healthy controls matched for age and gender. There is also a disease control of systemic lupus (SLE) where fatigue is a symptom but the illness is better illness, along with alternately diagnosed Lyme syndrome (ADCLS) patients.

The latter is partly due to funding but also that the study's collaborators at University of California, San Francisco, were already working on the Lyme disease gene expression signature. It has a similar phenotype and disability as CFS/ME, so is useful to compare and contrast. The study design is shown in figure 1.

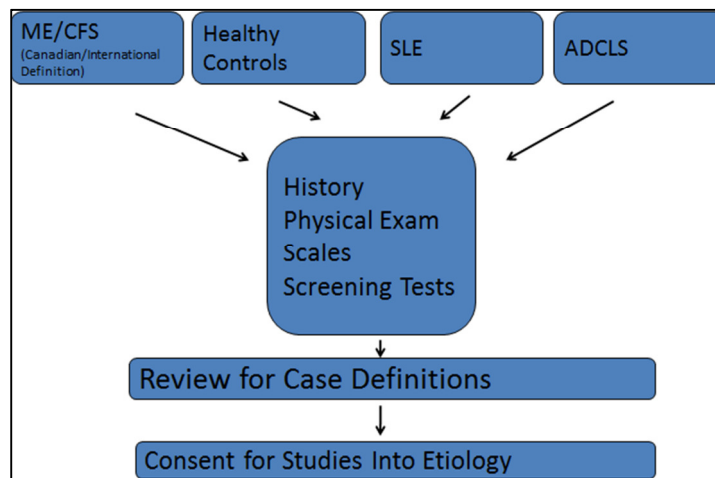


Figure 1: UBC Complex Chronic Disease study design

Two methods papers have been published, along with two minor reports on sub-maximal exercise testing and functional scales. The major descriptive paper was published in the journal *Clinical Infectious Diseases* in 2015. A metagenomics paper and a gene expression paper are currently in review by journals, and the immunosignature work would be presented in full at the IACFS conference in October.

Table 1 shows the age- and gender-matching in the study. Gender-matching was difficult in the Lupus group, and the BMI was greater in CFS/ME patients than in other groups.

	CFS (N=25)	Health y (N=25)	Lyme (N=13)	Lupus (N=11)	P CFS vs. Health y
Age	54 (48;62)	53 (49;63)	45 (40;52)	51 (32;62)	0.9
Gender (male)	4 (16%)	4 (16%)	3 (23%)	0 (0%)	0.6
BMI	26 (24;30)	22 (20;25)	22 (21;27)	22 (21;27)	0.01

Table 1: Characteristics of participants

Common symptoms and putative triggers are shown in Table 2. As shown in the highlighted figures, the symptoms of ADCLS and CFS/ME overlapped so much that 85% (N=11) of the ADCLS patients met the Fukuda CFS/ME definition; very few of them had the tick exposure and skin rash. Prof Patrick suggested that this could mean many people who have been given a diagnosis of chronic Lyme are misdiagnosed and actually have CFS/ME.

Group	Healthy (N=25) (N (%) or Median (IQR))	CFS (N=25) (N (%) or Median (IQR))	ADCLS (N=13) (N (%) or Median (IQR))	P CFS vs. Healthy	P ADCLS vs. Healthy	P CFS vs. ADCLS
Fatigue	4 (16%)	25 (100%)	12 (92%)	<0.0001	<0.0001	0.3
Post-exertional fatigue	2 (8%)	25 (100%)	11 (85%)	<0.0001	<0.0001	0.1
Non-refreshing sleep or sleep disturbance	8 (32%)	25 (100%)	12 (92%)	<0.0001	0.001	0.3
Pain or headache	15 (60%)	25 (100%)	13 (100%)	0.001	0.008	1.0
Neurological/Cognitive dysfunction	1 (4%)	25 (100%)	11 (85%)	<0.0001	<0.0001	0.1
Swollen joints	1 (4%)	8 (32%)	7 (54%)	0.02	0.001	0.3
Painful joints	7 (28%)	17 (68%)	12 (92%)	0.01	<0.0001	0.1
Meeting Fukuda CFS Definition	0 (0%)	25 (100%)	11 (85%)	<0.0001	<0.0001	0.1
Viral illness	N/A	11 (44%)	7 (54%)	N/A	N/A	0.7
Bacterial infection	N/A	4 (16%)	3 (23%)	N/A	N/A	0.7
Tick bite	N/A	4 (16%)	2 (15%)	N/A	N/A	1.0
Skin rash	N/A	2 (8%)	2 (15%)	N/A	N/A	0.6

Table 2: Common symptoms and putative triggers

The SF-36 Physical Health and Karnofsky functional scale scores show a commonality between CFS/ME and ADCLS, as can be seen in Figure 2. A score of under 60 on the Karnofsky scale is a level of disability that precludes work for most people, and these were not even severe CFS/ME patients but those well enough to attend the clinic, so it is very disabling even in mild and moderate CFS/ME. With the SF-36 Mental Health Score, there was no real distinction between the illness groups and the healthy control group, so poor mental health was not a factor that distinguished them.

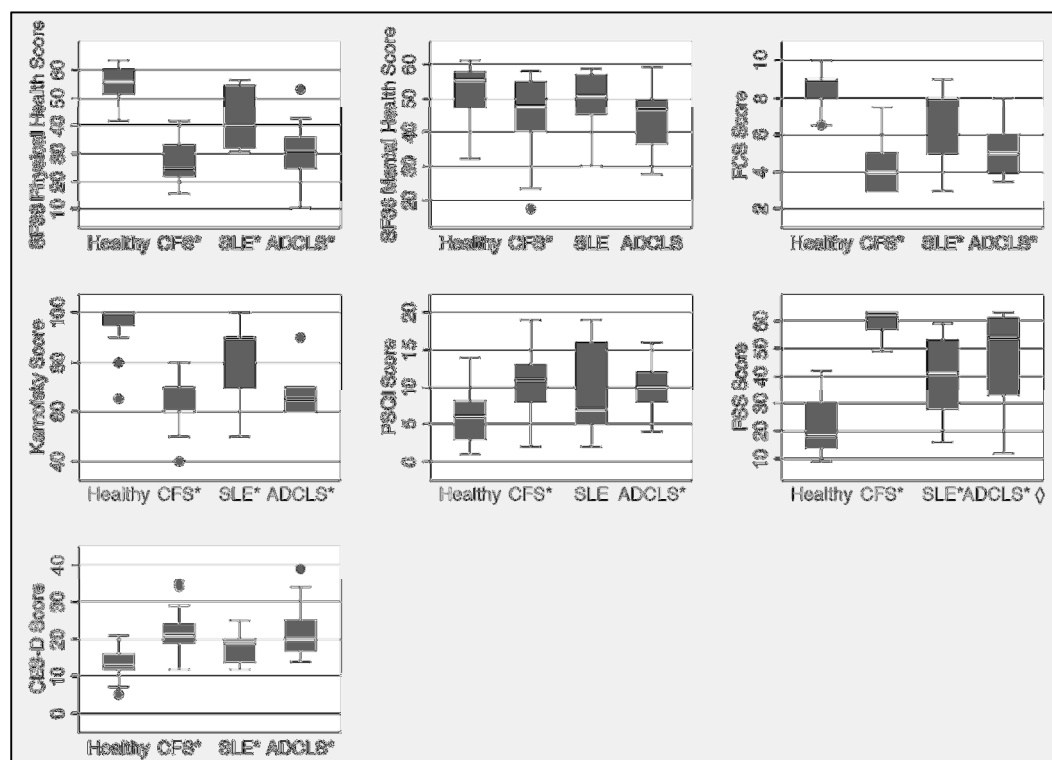


Figure2: Performance on functional scales by group

None of the subjects in the study, including those with a Chronic Lyme diagnosis, were positive for Lyme, as can be seen in the highlighted row in Table 3.

Group	Healthy (N=25) (N (%) or Median (IQR))	SLE (N=11) (N (%) or Median (IQR))	CFS (N=25) (N (%) or Median (IQR))	ADCLS (N=13) (N (%) or Median (IQR))
CD 57	5.5 (2.2;8.6)	2.3 (1.7;6.8)	5.0 (2.9;7.3)	5.1 (2.9;7.9)
CD 57 Absolute	0.10 (0.05;0.14)	0.04 (0.02;0.08)	0.09 (0.06;0.10)	0.10 (0.07;0.16)
Reference Lyme serology (Reactive)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
C6 peptide IgG Reactive	0 (0%)	2 (18%)	0 (0%)	1 (8%)
C6 Peptide IgM Reactive	0 (0%)	2 (18%)	0 (0%)	1 (8%)
<i>Anaplasma phagocytophilia</i>	2 (8%)	1 (9%)	1 (4%)	2 (15%)
<i>Ehrlichia chaffeensis</i>	0 (0%)	1 (9%)	0 (0%)	1 (8%)
<i>Rickettsia rickettsia</i>	0 (0%)	1 (9%)	0 (0%)	0 (0%)
<i>Coxiella burnetti</i> Q fever Phase I IgG	0 (0%)	0 (0%)	1 (4%)	0 (0%)
<i>Coxiella burnetti</i> Q fever Phase II IgG	0 (0%)	0 (0%)	1 (4%)	0 (0%)
<i>Bartonella henselae</i> *	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Babesia microti</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)
<i>Francisella tularensis</i>	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Table 3: Laboratory results

Hornig and Lipkin had a very large cytokine panel, whereas the UBC CCD study could only measure eight or 10 and found no significant difference in cytokines in the peripheral blood. A factor that could explain the differing results is that Hornig and Lipkin's mostly studied people in the first three years of their illness.

People with CFS/ME and people with alternately diagnosed Lyme disease have a similar or identical phenotype in this study and people are sick enough that it is worthwhile trying to better understand the causes.

A sub-study of sub-maximal exercise testing

Maximal cardiopulmonary exercise tests (CPET) hold promise but frequently result in symptom flare. A sub-study was set up to establish whether sub-maximal testing would be as useful to find out what was going on with tissue oxygenation and offloading of oxygen at tissue level, without inducing a symptom flare if possible.

A study of 16 people with CFS/ME and 14 healthy controls, all female and age-matched within five years, was carried out in conjunction with the Metabolic Diseases Unit. The participants were screened for mitochondrial disorders.

The participants undertook a handgrip exercise test of 30 contractions per minute for three minutes at 40% maximal voluntary contraction. Exertion was measured by recording both a modified Borg's rating of perceived exertion and objective measures using the machine. Muscle oxygenation and haemodynamics of the wrist extensor and flexor muscles were monitored using Near Infrared Spectroscopy (NIRS) to generate information on the changes in oxyhaemoglobin and deoxyhaemoglobin (HHb).

Univariate analyses were performed using a non-parametric Wilcoxon rank-sum test and multivariate analyses were performed using linear regression. Table 4 shows the results, comparing CFS/ME cases with controls.

	CFS/ME (N=16) Median (IQR) or N (%)	Controls (N=14) Median (IQR) or N (%)	P
Age	55 (49;65)	54 (47;63)	0.7
Gender - female	16 (100%)	14 (100%)	N/A
BMI	24.2 (21.4;30.8)	20.8 (19.9;24.6)	0.06
Karnofsky	60 (60;70)	100 (90;100)	<0.0001
Hb	133.5 (129;139)	132 (123;138)	0.5
Acyl carnitine	11.5 (8;14.7)	11.3 (8.7;13.3)	1.0

Table 4: Comparison of CFS/ME cases and controls

When focusing on area under the curve (AUC) in the graph of the results shown in Figure 3, HHb was significantly lower for the CFS/ME cases compared to controls (P=0.004). CFS/ME patients are getting less deoxyhaemoglobin detected, so less off-loading of oxygen to the tissues throughout the test.

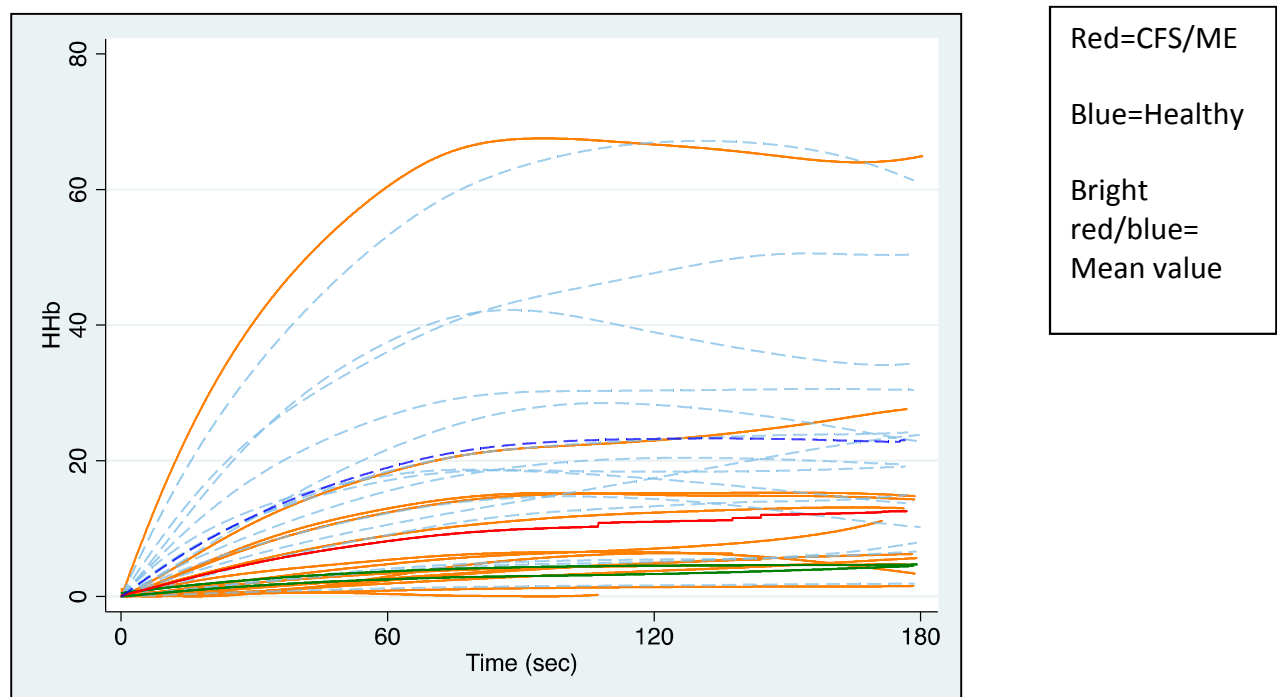


Figure 3: HHb over time.

However, when the measured exertion was examined it became apparent that the CFS/ME patients were not able to use as much force as the healthy controls during the test, as seen in Figure 4. The use of oxygen is in proportion to the effort used, so the results needed to be adjusted for this.

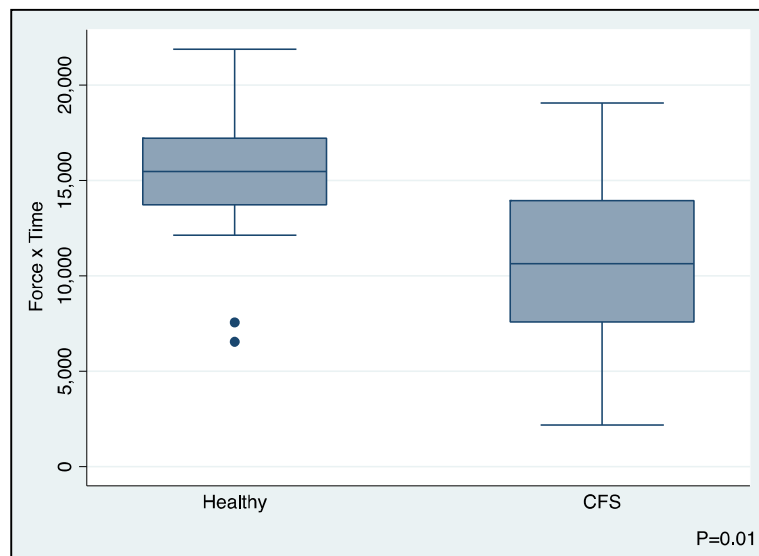


Figure 4: Measured effort

After adjusting for measured effort (newton-seconds), as in Figure 5, there was no significant difference in AUC of HHb between cases and controls (P=0.15). Nor was there when further potential confounders (age, BMI, haemoglobin and ferritin) were considered (P=1.0).

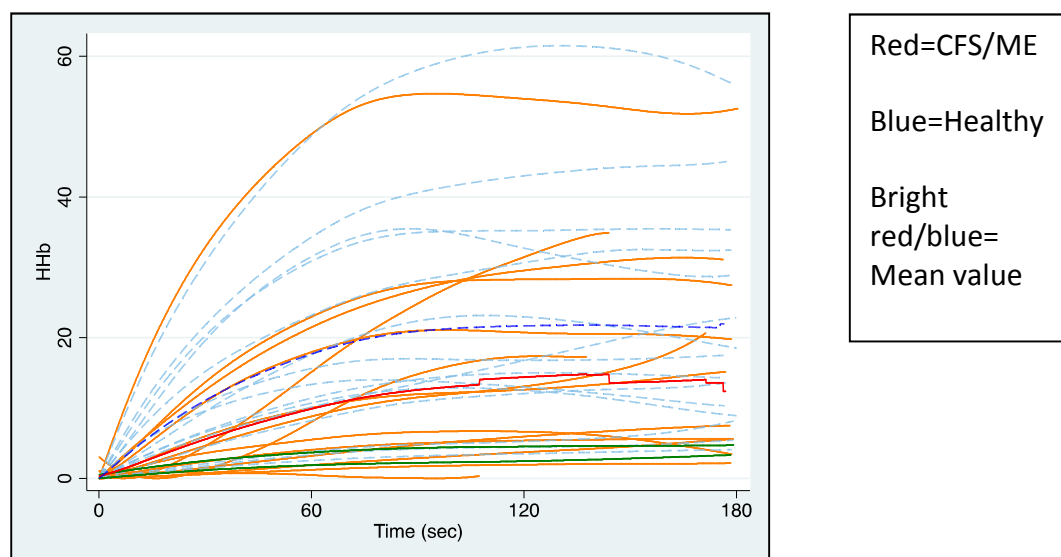
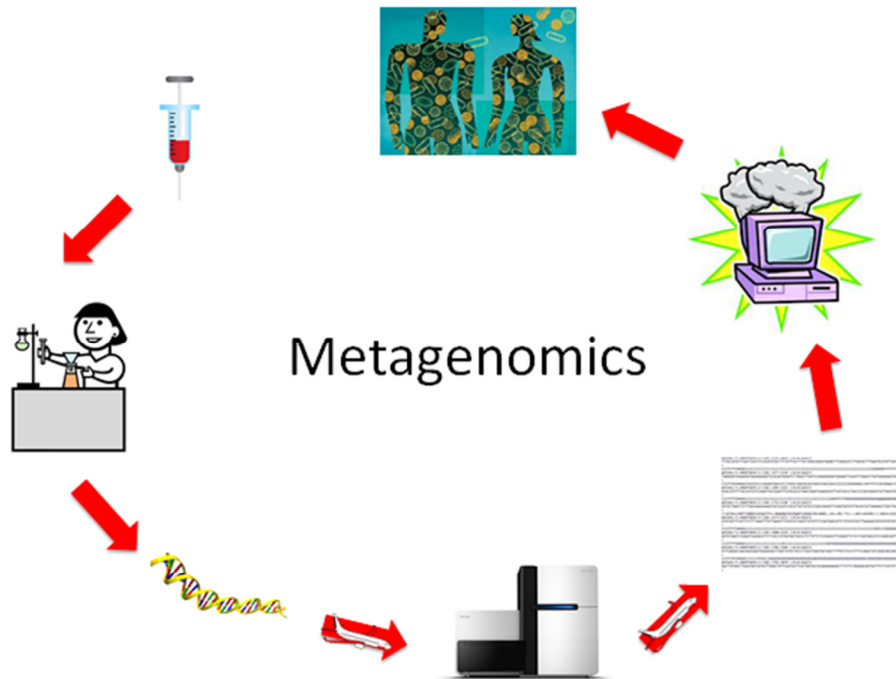


Figure 5: Analysis adjusted for measured effort

In conclusion, said Prof Patrick, the sub-maximal exercise testing procedure did not clearly distinguish between CFS/ME cases and healthy controls. A subset of 19% of CFS/ME patients met criteria that prompted further testing for mitochondrial disease – the original purpose of the test.

Metagenomics



Moving onto metagenomics, Prof Patrick illustrated the process with the diagram above. The **syringe** represents sample collection, in this case plasma from the four study groups.

The **laboratory** represents the lab work performed, ie. extracting DNA from the plasma and preparing it for input into the DNA sequencer, in a process called library preparation. One problem, said Prof Patrick, is that there are quite low levels of microbial DNA in plasma samples so they had to amplify up the DNA before library preparation, using random primers, which randomly amplify up DNA sequence in the sample.

The **DNA** shows that the team eventually gets DNA from the samples. Prof Patrick explained that the next arrow is overlaid with an aeroplane because the DNA is flown to McGill University in Montreal for sequencing.

The **Illumina HiSeq** the next-generation sequencing platform used in the study. It generates multiple short copies of all the DNA present in a sample (~75-250 nucleotides long) known as “reads.”

The next image shows a copy of a piece of a fastq file. Each four lines represents a piece of **DNA sequence** and its associated quality, ie a read. In this case, there an average of 13 million reads for each sample, and each read is 100 DNA bases long.

The **computer** represents all of the bioinformatics analysis undertaken by Prof Patrick, which is explained in the next slide.

The **bodies** represent the results of the analysis, where reads are matched to a reference to determine their taxonomy and/or function. And comparison of reads between individuals with disease and healthy controls may find associations.

The full paper is in review but Figure 6 shows the preliminary data for bacteria and Figure 7 for viruses.

Each line is a study participant and there is no clear clustering between the study's groups. This is the 25 most prevalent bacteria, and by no means all identified. The purpose of the negative control (which is just DNA-RNA-free water subjected to exactly the same laboratory and bioinformatics pipeline) is to remove things that might be false positive because they are present in the negative.

These likely arise due to contamination of the reagents. By eye, there is no discernible difference between the CFS/ME and healthy controls. These results are generated using RAPSearch2. Before making conclusions about anything deemed present by RAPSearch2, BLASTn validation was performed.

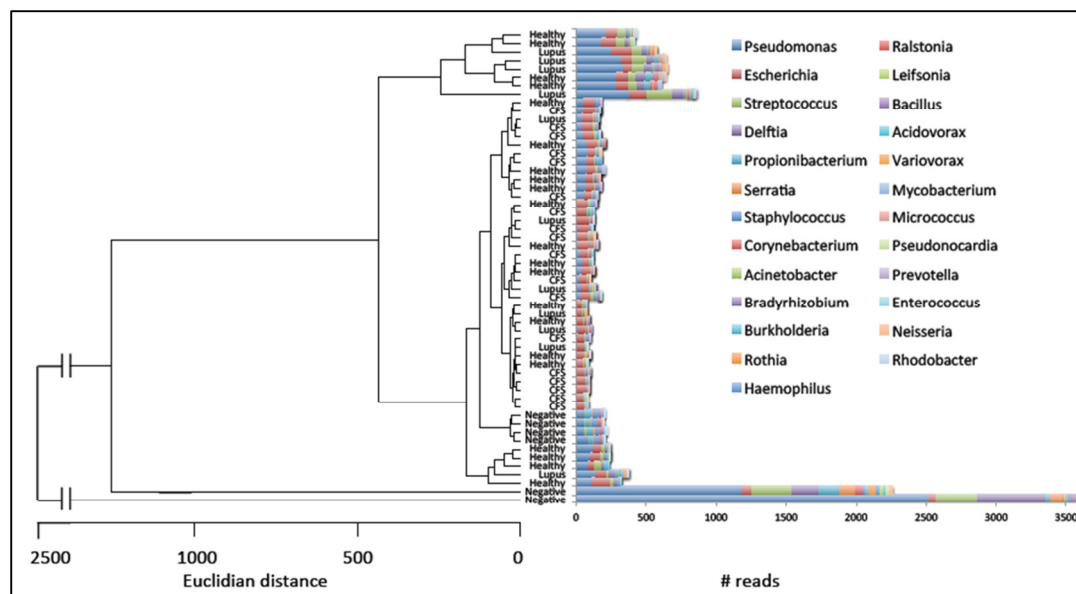


Figure 6: Preliminary data shown at IDSA - bacteria

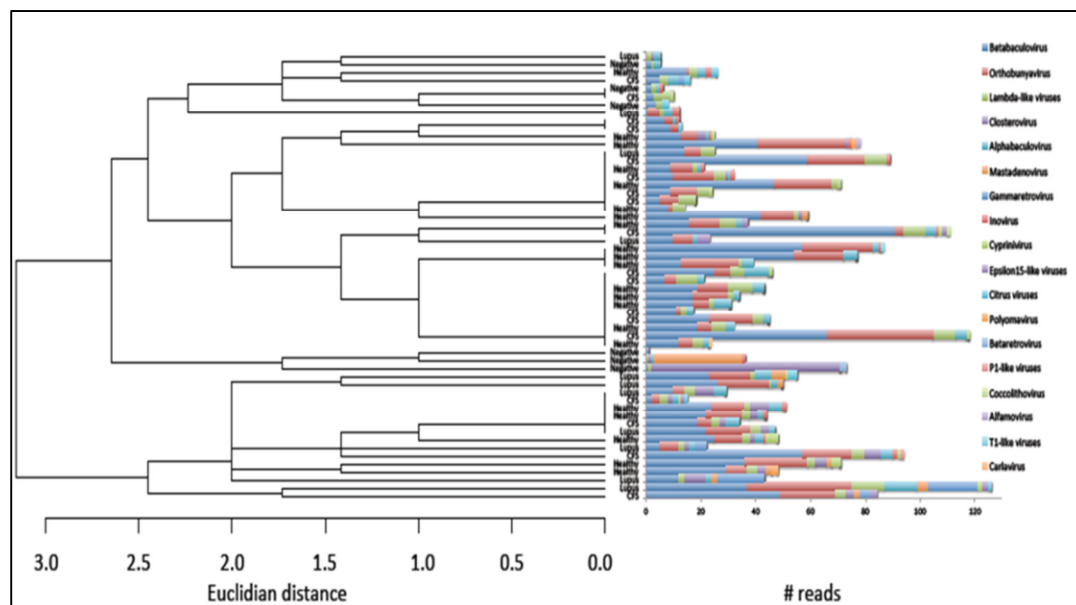


Figure 7: Preliminary data shown at IDSA - viruses

The paper in review reports that there were no large differences between the viral and bacterial content of blood between people with CFS/ME and healthy controls. However, this is not the end of looking for infectious disease in CFS/ME as every tissue in the body has not been investigated.

Early report on gene expression

The RNA quality is very good, and SLE is a good positive control. There were relatively few differentially expressed genes between CFS/ME, ADCLS, and healthy controls. No overlap between published profiles on Lyme disease and ADCLS, and no clear correlation with core co-variables (fatigue severity, etc) were found.

More analysis is underway to see if there are any correlations between the transcriptomics and other co-variables.

Potential value of exercise testing in CFS/ME

The aim is to be able to use exercise testing for the identification of discrete physiological sub-types within CFS/ME using a standardized, measurable “experiment” to explore gene expression during any resulting symptom flare.

Participants with fluctuating CFS/ME were willing to recreate their post-exertional malaise for the research team to study, but it was not possible to include patients with severe CFS/ME for obvious reasons.

CFS/ME and “deconditioned controls”

The results of an CPET/transcriptomics study on pre- and post-exercise, funded by National Institutes of Health and ME Research UK, are currently being analysed

Immunosignature work

There are high throughput technologies in terms of genomics which have revolutionised approaches to gene sequencing. However, in terms of defining what is happening with antibodies, it has been hard to look at the totality of antibody expression.

Stephen A Johnston at Arizona State University has developed peptide-based micro arrays that have 125,000 peptides in one version and 330,000 peptides in another, on a chip. These are randomly selected from antigen space but it gives a good broad overview of where antibodies are being expressed. These micro arrays have successfully been used to make early diagnosis of Valley Fever and also to interrogate vaccine responses and cancer research to investigate antibodies associated with specific malignancies.

Professors Patrick and Johnston have begun looking at antibodies in CFS/ME and some patterns are evident between CFS/ME and controls, so there is some potential to define a specific disease signature. Prof Patrick is also working with Fluge and Mella on specific signatures for responders to rituximab, though these early findings are being considered as hypothesis-generating. Funding is needed to test Fluge and Mella’s entire phase III rituximab trial participants to prove or disprove the hypothesis.

Where does causal inference need to go next?

There are now several cross-sectional omics studies and what is needed next are large cohort studies and nested case-control studies, explained in Figure 9. The UK has good cohort studies and the best biobank linked to them.

Large population-based cohorts combined with biobanked blood specimens do not necessarily need to have been specifically set up to study CFS/ME; they could have been set up to study cancer, as in a cohort of 300,000 healthy people in Canada. During the three years since the cohort was established, some of these healthy people will have gone on to develop CFS/ME, so their biobanked specimens can be studied alongside a sample of those who did not.

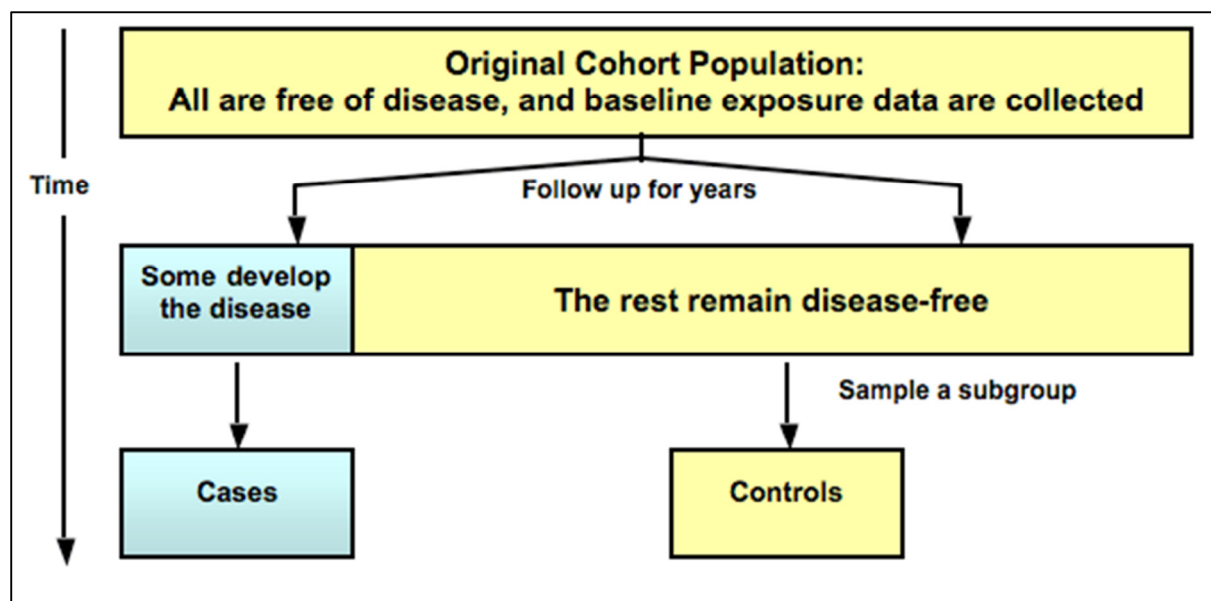


Figure 9: The process of nested case-control studies.

Source: <http://cliomods.stanford.edu/trailmaps/design/design/nestedCase-Control/index.html>

As an example, for the metabolomics profile for CFS/ME found by Naviaux's team, we can see whether the results are actually from inactivity due to the illness or may indeed have preceded or coincided with symptom onset.

Platforms currently showing promise include cytokine networks metabolomics and immunosignature. Some cohort studies are already up and running, and studies within them may be most practical if we join forces with others, concluded Prof Patrick.

All in at the Solve ME/CFS Initiative
Dr Zaher Nahle, Solve ME/CFS Initiative

Key point summary

- The Solve ME/CFS Initiative has two functions: facilitating and supporting the research of others, and generating research in-house.
- The Institute of Medicine report had a “domino effect” in terms of increasing interest in CFS/ME research.
- Misperceptions about the illness are common, despite robust evidence. Patients must be empowered to have control over their own data.
- Targeted research is essential to move the field forward, working in collaboration with others.

Dr Nahle said he would offer two presentations in one, speaking about the work of his organisation and its perspective on the CFS/ME research landscape. “I have a section called the No Spin zone, covering misconceptions and misperceptions about the illness, that I would like to share with you,” he said.

The Solve ME/CFS Initiative (SMCI) has two functions. One is facilitating and supporting the research of others, through a variety of mechanisms including its grant making programme (the Ramsay Awards), biobank and patient registry services, and medical webinars for influencers and thought leaders.

This year it is also embarking on a research-generating effort, taking on in-house projects in collaboration with others. Dr Nahle explained he would talk specifically about three initiatives, focusing on pathways and biomarker discovery, and also SMCI’s work with Memorial Sloan Kettering Cancer Center on potential drug screening.

“I want to give as much information as I can, seeking partnerships, synergies and potential collaborations,” he said.

SMCI is non-profit organisation dedicated solely to the cause of CFS/ME, and has been in business since 1987. It engages in all aspects from advocacy to research – but research continues to the core of its work. All its directors have experience of the illness and its scientific board counts among them world leaders in CFS/ME, medicine and technology. Its President Carol Head, said Dr Nahle, is very committed to the cause.

Giving an overview of key events in the history of CFS/ME, he highlighted that this is not a new disease: “Darwin himself could have been diagnosed, according to the Fukuda criteria.” Outbreaks have been reported in the UK, dating back to the 1930s, with confusion over the cause. In the US, attention started to be paid when outbreaks occurred in upstate New York and Nevada. The Centers for Disease Control and Prevention (CDC) commissioned a working group to study this, resulting in the Fukuda criteria, published in 1994, which was never meant to be a clinical criteria.

A variety of other criteria have been put together by similar assemblies, such as the Canadian Consensus Criteria (2003), and the Institute of Medicine’s report (2015). The latter

was significant, says Dr Nahle, because “it was the first time the medical establishment had decried the lack of investment in CFS/ME.”

Dr Nahle described how he made a graph by plotting the number of publications on certain illnesses as a function of time. It shows that publications about CFS/ME were on the rise in the 1980s, consistent with the attention paid to the outbreaks.

However, when the graph is adjusted to add other illnesses, the considerable knowledge gap on CFS/ME is revealed – see Figure 1. Furthermore, when you look at funding per patient for CFS/ME in the US, the figure amounts to less than \$2 a year.

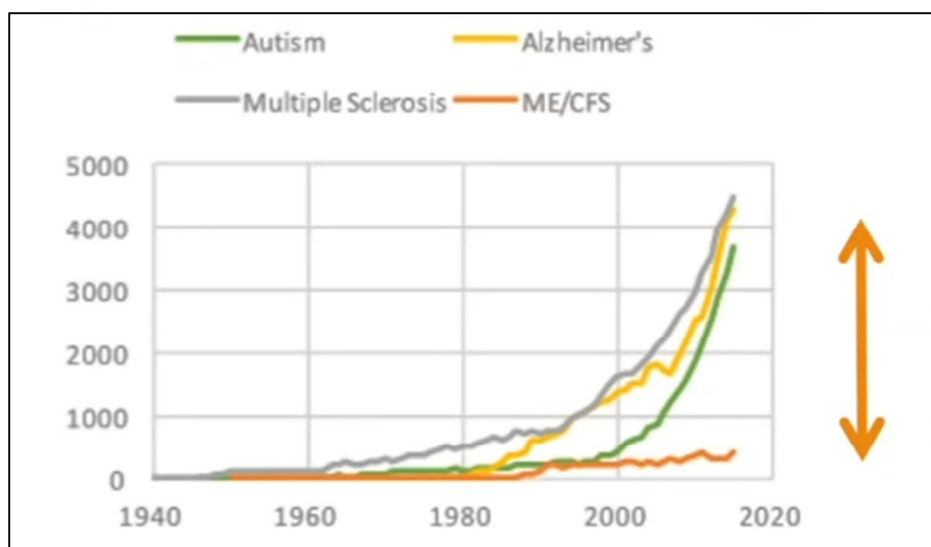


Figure 1: Number of peer-reviewed research publications by disease, 1940 to present

Dr Nahle was moved to thank all the scientists, institutions and collaborations, including the CMRC, who move CFS/ME research forward despite the lack of funding. “Things are beginning to change now,” he said, partly thanks to the Institute of Medicine report, commissioned by a number of federal agencies. “What we are seeing now is a domino effect in response to the report.”

He spoke about actions taken by the US National Institutes of Health (NIH) in this respect, along with initiatives at the Centers for Disease Control and Prevention. This includes CFS/ME being included in a recent Grand Round (the mechanism by which it highlights focus on key issues and challenges related to a specific health topic) and the establishment of a Technical Development Work Group, to which Dr Nahle belongs, to consider recommendations made by the Institute of Medicine report.

Furthermore, the US Agency for Healthcare Research and Quality has issued an unprecedented addendum to its 2014 evidence review on the suitability and efficacy of CBT and GET for CFS/ME.

Combined with the potential of the work being done in the -omics field, there are reasons to be hopeful.

No Spin zone

Patients are frequently being told they look normal, said Dr Nahle. But as the study results in Figure 2 show, CFS/ME patients are more disabled than people with cancer, diabetes, stroke and many other serious diseases.

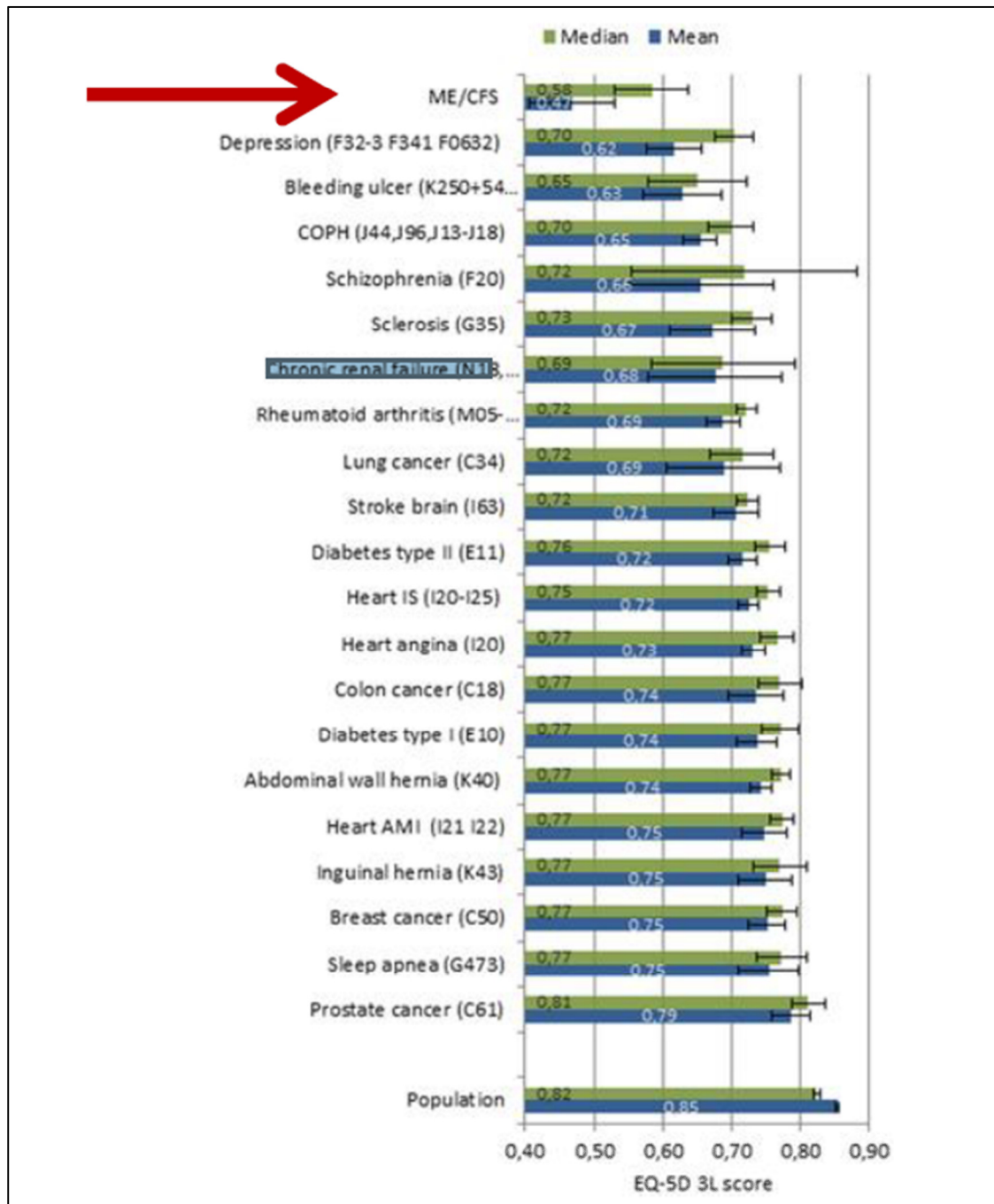


Figure 2: Quality of life for patients with CFS/ME ranks the lowest when compared to other devastating diseases. Source: Hvidberg et al (2015) The Health-Related Quality of Life for Patients with CFS/ME. PLoS ONE 10(7): e0132421. doi:10.1371/journal.pone.0132421

The second part of Dr Nahle's No Spin zone is that CFS/ME is caused by depression or anxiety, said Dr Nahle. But actually the opposite is true; depression and/or anxiety are the

most common emotional responses to medical illnesses so it's not surprising that many patients experience these. This is not a causality issue – quite the opposite (see Figure 3).


<div>This is caused by Depression or Anxiety</div>	
No Spin Zone	
The opposite	
Depression/Anxiety	ME/CFS
1- HYPER-cortisolism	1- HYPO-cortisolim
2- Enlarged adrenal glands	2- Reduced in size and function
2- Reduced serotonin	2- Abnormal increase in serotonin!
3- Anhedonia, guilt, lack of motivation	3- The opposite lack of energy not interest
4- Exercise improves states in depression	4- Exercise (if possible) causes PEM/crash

Figure 3: Depression and/or anxiety are the most common emotional responses to medical illnesses


<div>Your blood test came back normal!</div>	
No Spin Zone	
Differences in Blood using high tech analysis (2016/2015)	
1- Nuclear Magnetic Resonance: metabolic signature (2015 Metabolomics, Armstrong et al)	
2- Mass Spectrometry: metabolic signatures (2015 PNAS, Naviaux et al)	
2- Immune-profiling: distinct signatures (2015 Science Adv., Hornig et al)	
3- High-tech sequencing: Abnormal pathogenic features (2016 Microbiome, Giotaux et al)	

Figure 4: Differences in Blood using high tech analysis (2016/2015)

Thirdly, Dr Nahle highlighted how patients are frequently told that their blood test has come back normal. But his response is: what YOU measured in my blood came back normal. With more detailed analysis, there is much to discover, as illustrated in Figure 4. For example, Armstrong’s 2015 study identified a clear metabolic signature in CFS/ME patients.

Objectives and priority areas

Moving on to the work of the SMCI, Dr Nahle highlighted its objectives and priority areas, noting their similarities to those of the CMRC. These are:

- increase understanding of the molecular basis of CFS/ME
- identify a reliable biomarker and credible testing for CFS/ME
- develop effective treatment(s) for CFS/ME patients.

Within those objectives, it has established three key areas of focus: bioenergetics, immunity and inflammation, and neuroendocrine biology. “We know that, in science, the answers never come from where you expect,” said Dr Nahle. “So we are keeping an open mind about other areas as well. All are part of a continuum and very much interconnected.”

Looking at the philosophy of science, three research domains must come together if we are to fix complex problems. These are illustrated in Figure 5.

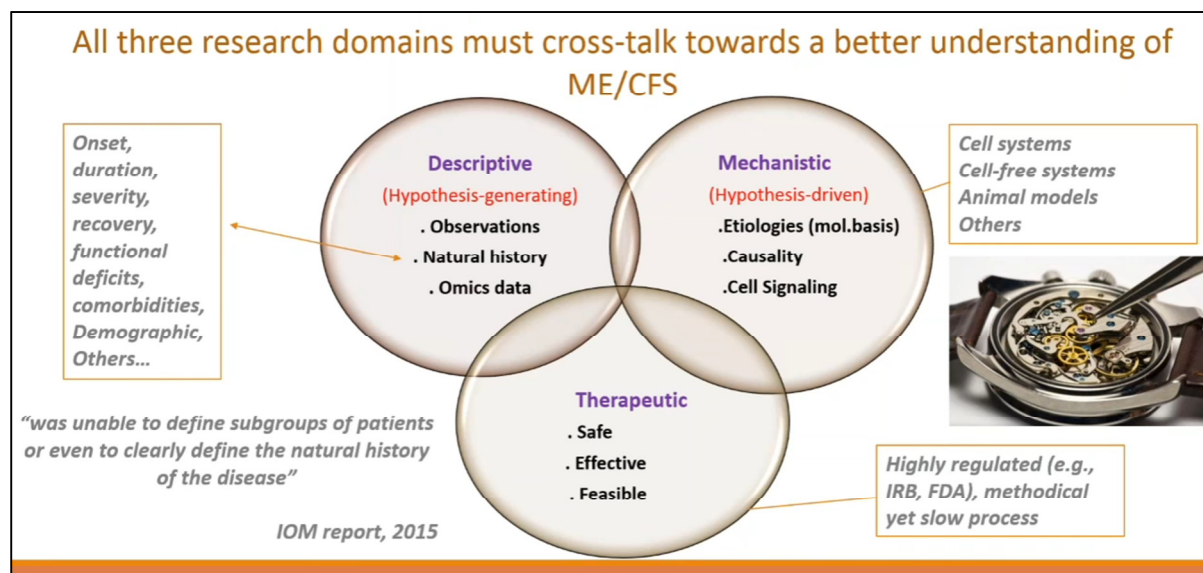


Figure 5: SMCI research philosophy

The first is descriptive research – surveys, -omics data – that helps you to prioritise and develop hypotheses; the natural history of the disease is a very important factor in this, an area of knowledge severely lacking in CFS/ME. The second area is mechanistic understanding, delving in to deep mechanisms about causality, aetiology and underlying signalling pathways. Both of these are very important in developing safe and effective therapies, the third area, through rational drug design

Ramsay Award programme

SMCI’s grant-making programme, the Ramsay Award, has already gone through two cycles in 2008 and 2012. The awards range in size from \$35-55,000 with the possibility of renewal based on results.

The programme received international submissions for this year’s cycle, with only 40% coming from the US: the rest were split between the UK, Australia and Germany. A high number of applications came from female scientists, and 40% of were from early-stage career researchers. “We have seen a diversity in topics and background that reflects the pleiotropic complex nature of CFS/ME,” said Dr Nahle. *[Addendum: SMCI announced the latest recipients of its Ramsay Award programme on its website in December 2016.]*

Related to this, the organisation launched the meetME Travel Award programme, aimed at facilitating participation of underrepresented groups in meetings and conferences focused on CFS/ME. The first was awarded to a student in the UK.

Moving on to the SMCI biobank, Dr Nahle explained that they aimed to reduce the barriers faced by scientists when it comes to acquiring samples for study. The capacity of the biobank is being increased by the addition of further data, and the only criteria for those who apply to use the biobank is that they use rigorous science. Examples of work being undertaken at the moment include Dr Jay Chung at the National Institute of Health, looking at biomarkers; and an auto-immunity study at the University of Vermont.

Dr Nahle announced the imminent launch of a 21st century patient registry. This is separate from the biobank, and offers great value, because it tells us about the natural history of the disease: diagnostic data, prognosis, quality of life, disparity data, drug interactions. This can help refine sub-groups in study design, and is key in supporting essential longitudinal studies to determine patterns and progression of disease.

To launch a patient registry effectively, you need the right platforms and the right number of patients to generate reliable data. Submitting applications to the Robert Wood Johnson Foundation and the Genetic Alliance, the SMCI received initial seed funding to set up the platforms it needed.

Every year for the past 12 years, the US government's Health and Human Services committee on CFS/ME (the CFS Advisory Committee or CFSAC) has made a recommendation that a resource must be set up in order to better understand the natural history of the disease. The IOM report was unable to define sub-groups of patients, and this is a major barrier to progress. For these reasons, the SMCI set up its patient registry, and is seeking collaboration with others to support this work on an ongoing basis.

The platform itself is in process of being set up, and will be a simple interface where patients and professionals can submit information. There will be a premium on privacy. "You must empower patients to have control over their own data," said Dr Nahle. Figure 6 shows the results of a survey of thousands of patients in the US, which looked at data sharing and informed consent. Most patients fall in the yellow sphere, where they want each study using their data to contact them in advance to get their specific consent. Fewer than 16.5% did not want their data used at all.

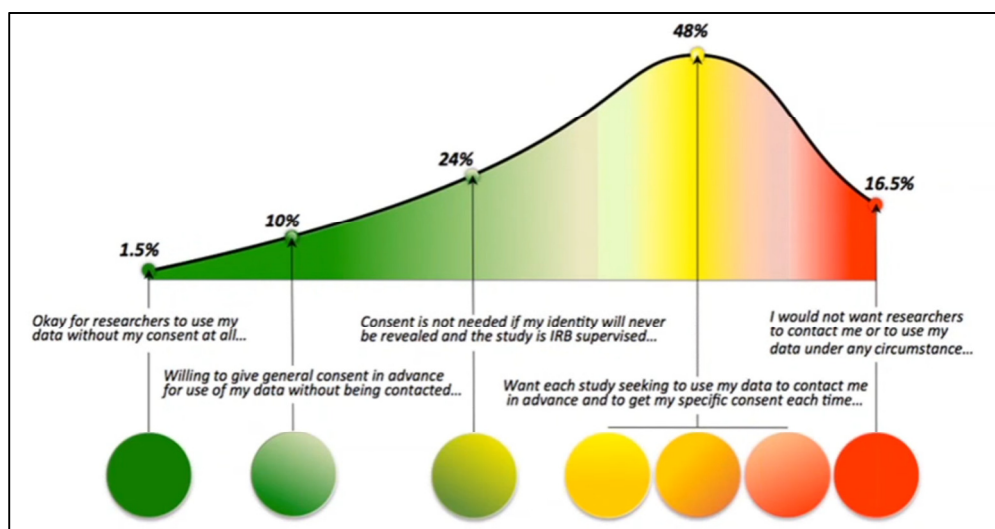


Figure 5: Results of patient survey on how data is used

Using this scale, patients contributing to the register are asked to indicate how they would like their data to be used. Other features of the registry include links to large health record systems, and compatibility with the Precision Medicine million-person study currently ongoing in the US.

Medical education and other projects

Another key segment of the SMCI's work is medical education. The organisation invites public officials, for example from the CDC or NIH, to give updates; recordings are then shared publically on YouTube.

Dr Nahle then moved onto talk about his organisation's Targeted Initiative research programme, to "change the status quo and invest in areas that we think can change the field a little bit."

Focusing on three initiatives, the first is pathway and biomarker discovery, a collaboration with Dr Sue Levine and Prof Maureen Hanson.

Working in partnership with Metabolon, a metabolomics company, the team is studying the same patients involved in Prof Hanson's gut microbiome project (*Genome Medicine*, 2015), which means they are well characterised. "It's hypothesis driven but also a hypothesis-generating combination," he said.

The second project focuses on senescence, the phenomenon by which cells lose their capacity to function. This is a collaboration between SMCI and Dr Sheila Stewart, Washington University and Dr Masashi Narita, University of Cambridge, both experts in this area. Senescence is the phenomenon by which cells lose their capacity to function. It is controlled also by DNA damage response and other factors, such as telomere dysfunction and oxidative stress, and it's known that many of these happen in CFS/ME. In fact, an analysis of reduced telomere length in CFS/ME has been published by the CDC's Dr Beth Unger (*Faseb Journal*, 2016) and the SMCI is collaborating with the CDC on this project.

The third big data project is a functional genomics study to uncover potential drugs screening in CFS/ME, a project in collaboration with Memorial Sloan Kettering Cancer Center and Dr Scott Lowe, Chair, Cancer Biology and Genetics Program. This uses using platforms and technologies that will allow the team to look at phenotypes and certain changes in the potential of cells from patients, such as the increase of the cytotoxicity potential in blood cells.

This type of analysis requires collaboration and considerable groundwork in the laboratory, in a long and time consuming process. "But I'm very excited about this kind of screening, particularly because they already have the compounds on the market that correspond to these druggable targets," says Dr Nahle. "So if we find something we can rapidly accelerate it."

Dr Nahle closed his presentation by thanking all scientists working on CFS/ME and the patient community, "supporting us with money but also literally with blood."

States, traits and diagnosed conditions: implications for the design of population-based studies of human health and disease

Prof George Davey Smith, MRC Integrative Epidemiology Unit, University of Bristol

Key point summary:

- Virtually all medical conditions are directly part of a spectrum, or reflect a liability which lies along a spectrum.
- Wide inclusion criteria and large sample sizes are needed to remove selection bias from studies and reveal the whole picture.
- Subgroups within broad disease groups may reflect varying combinations of liability and specific exposures.
- Attempts to identify and dissect such subgroups should be carried out at the analysis stage, with replication.

Prof Davey Smith began by explaining that although he is not an expert on CFS/ME, he has experience of working on the design of large-scale biomedical epidemiological studies of other conditions, the principles of which are applicable to the type of CFS/ME study being discussed at the conference.

The normal and the pathological

The debate about whether health and disease are two discrete states goes back hundreds of years, but the view of Claude Bernard, who established the use of the scientific method in medicine in the 1860s, was that: *“Health and disease are not two essentially different modes as the ancient physicians believed and some practitioners still believe. They should not be made into distinct principles, entities which fight over the living organism and make it the theatre of their contest. These are obsolete medical ideas. In reality, between these two modes of being, there are only differences of degree: exaggeration, disproportion, discordance or normal phenomena constitute the diseased state.”*

This has become clearer in the studies of disease in the time since. Between the 1950s and 1970s, there was fierce debate central to British medicine, about what constitutes the difference between normal and pathological blood pressure. One view, by Baron Robert Platt was that hypertension is a distinct disease, separate from the rest of the population with normal blood pressure, having a bimodal distribution, shown in figure 1 as two peaks.

Contrary to this, Sir George Pickering proposed that instead, blood pressure is normally distributed, as in the bell curve in figure 1, and that blood pressure being defined as hypertension or not is arbitrary. The association of blood pressure to important cardiovascular events such as stroke or coronary heart disease is linear.

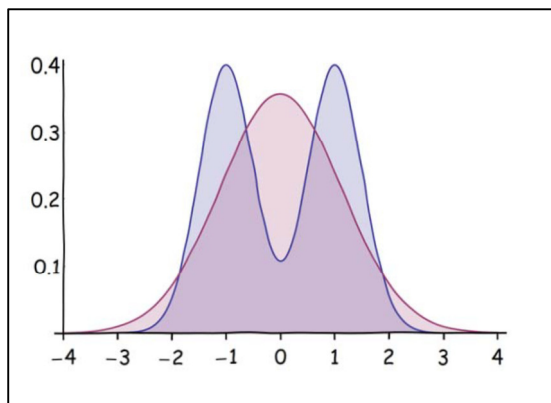


Figure 1: Platt versus Pickering

This debate raged in the pages of medical journals at the time, similar to the way that there are often heated debates now in the field of CFS/ME. The cause of these debates is often that data available at the time is rather limited and does not reveal a clear picture.

As shown in figure 2, it is possible to incorrectly interpret the curves as fitting Platt's hypothesis of having bimodal distribution, when in reality the distribution is normal with a slight tail, and the association with disease risk is continuous.

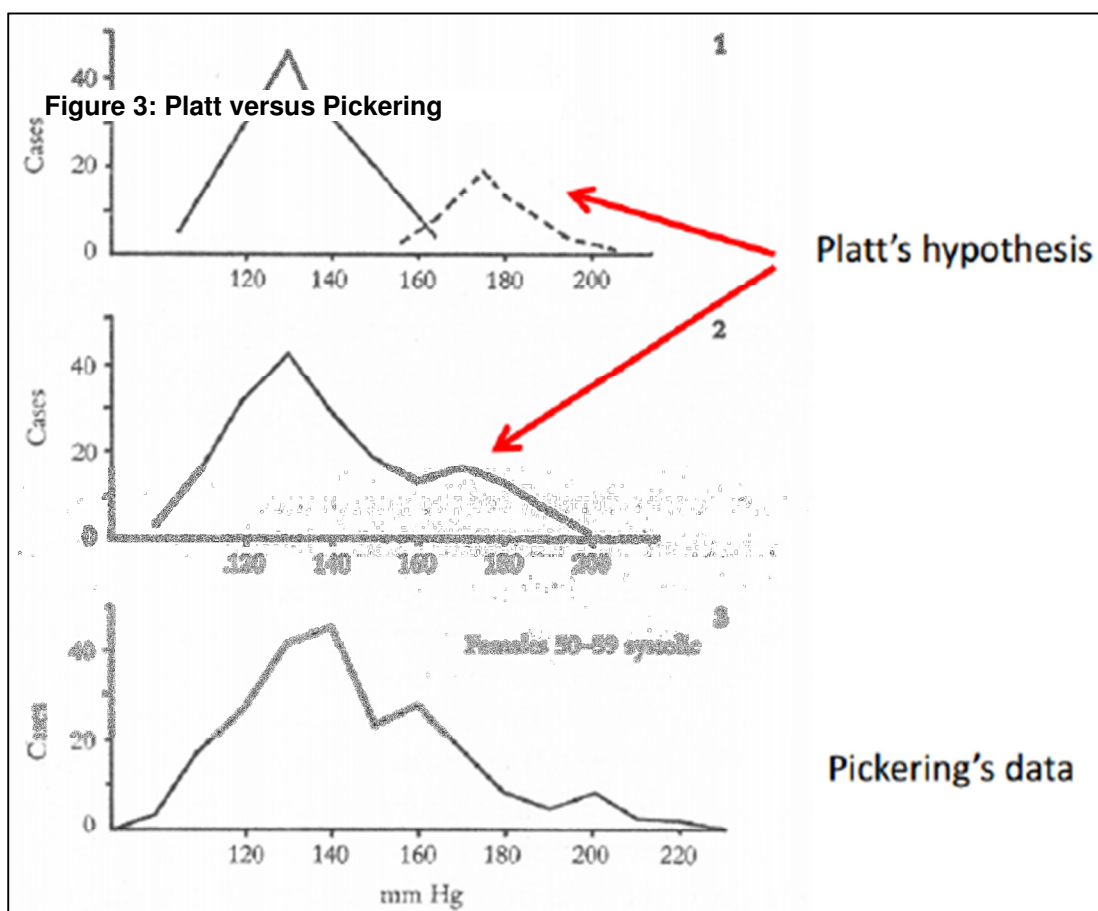


Figure 2: Number of cases against blood pressure (mmHg)

Now it is well established that blood pressure data support a continuity hypothesis. With different aetiologies feeding in to this normal distribution, it does not matter whether a person's blood pressure is raised from living on crisps or because there is a genetic tendency to have high blood pressure; both increase the risk of heart attack or stroke.

In figure 3, the distribution of blood pressure among those who went on to die of heart attack or stroke and the survivors shows considerable overlap, with a shift of the distribution to the right in those who succumbed .

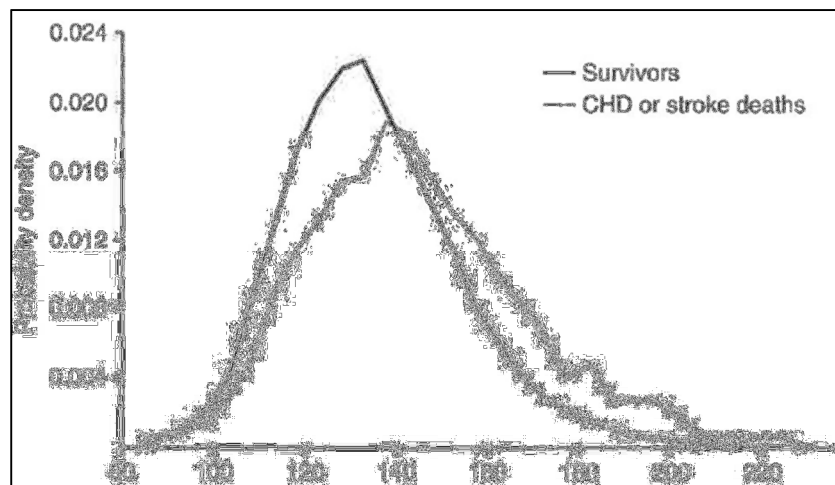


Figure 3: Distribution of systolic blood pressure (mmHg) in men who died from a heart attack or stroke compared with survivors. Rose D. *The strategy of Preventive Medicine* 1992

There are many other examples of this throughout medicine, such as glycated haemoglobin (HbA1c), which is an indicator of blood glucose levels, and increased risk of mortality (Khaw et al, 2002). Similarly, intraocular pressure and glaucoma (Leske et al, 2002), and bone heel ultrasound and fracture risk (Khaw et al, 2004) show these patterns.

Notion of liability

The notion of liability suggests that conditions have an underlying liability which is continually distributed. These models have been used in genetics since 1901, when Pearson introduced them. Figure 4 depicts liability as a normal distribution with the notion that individuals affected will be at the far right. However, anyone has the potential to develop the disease, but the probability increases as liability increases.

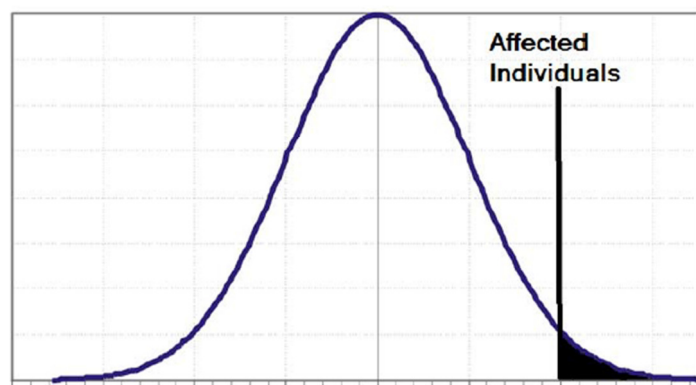


Figure 4: Standard liability model

Implications

Categorical outcomes (eg. heart attack, death, stroke) can have continuously distributed underlying liabilities with increasing probability of transition into disease state with increasing level of liability. Understanding contribution to liability provides inroads into understanding disease causation and potential prevention. Such models apply, to differing degrees, to virtually all medical conditions.

Considering autism where, in extreme forms, there is no doubt about the diagnosis, there is clear polygenicity (the contribution of many genetic variants) to what becomes the same diagnosed condition.

Looking at traits related to autism, such as social, communication and daily living skills, they are continuously distributed in the population. The Simons Simplex study looked at single cases of autism in a family; their siblings who did not have the diagnosis were also recruited as controls.

The siblings share genetics with the cases, and have a higher distribution of the traits when compared to the general population. There is a considerable overlap between cases and control siblings, as shown in figure 5. Although it can seem like a distinct condition in some cases, there is some arbitrariness in saying where the line is drawn between an autism diagnosis and no diagnosis.

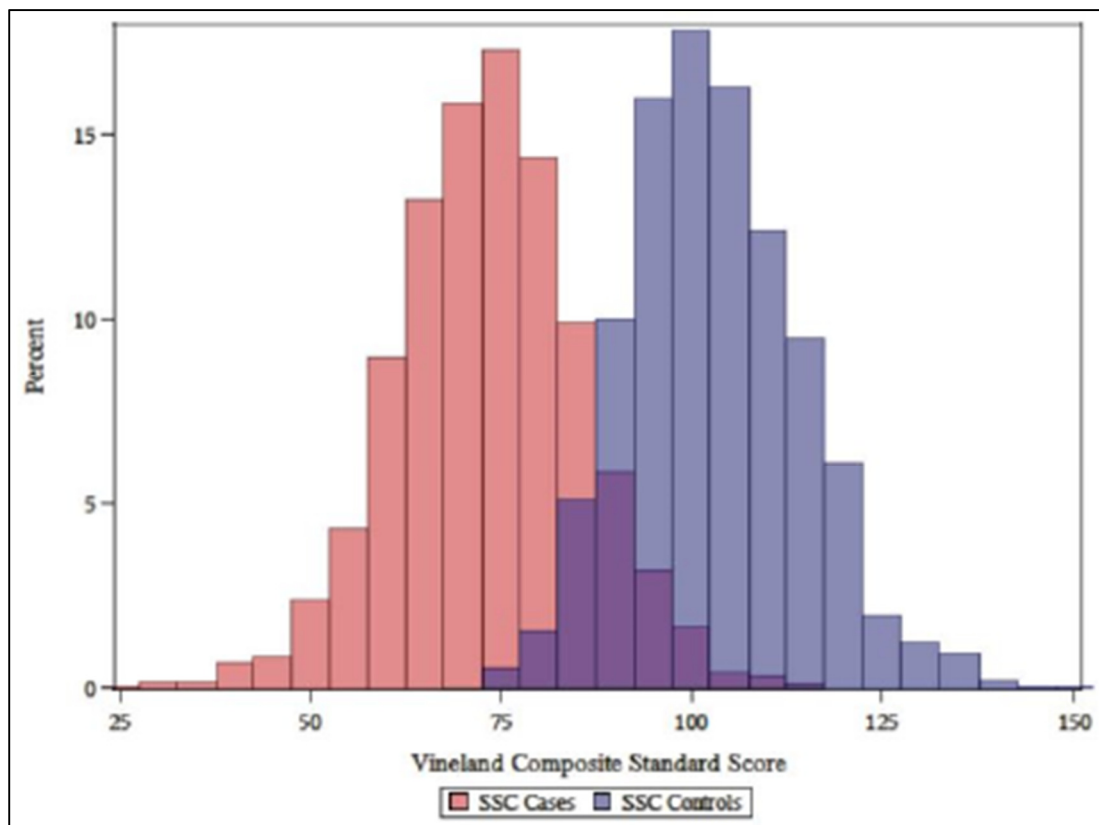


Figure 5: Vineland social, communication and daily living skills in cases and unaffected siblings in the Simons Simplex Collections (SSC). Robinson et al. Genetic risks for autism spectrum disorders and neuropsychiatric variation in the general population. Nature Genetics 2016; 48:552-555

There are not many, but some conditions are exceptions and aren't on a continuum. The disease is either there or not, such as Rabies and Huntington's disease, which are examples of fully penetrant infections on monogenic conditions.

If a condition has many underlying distributed liabilities, then using particular characterisations or categories of characterisations as inclusion criteria for a study, has implications for what the study can show. Mainer ET al's 2016 discussion of diagnoses in overlapping pain conditions shows that by having very proscriptive criteria for inclusion can mean that misleading conclusions are drawn.

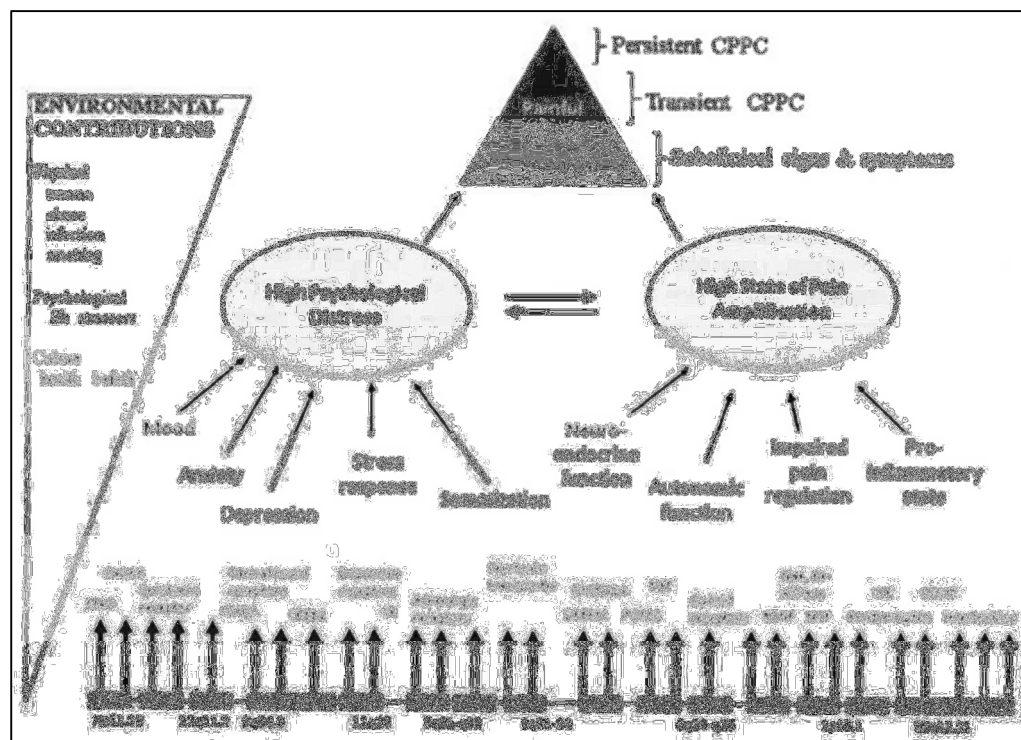


Figure 6: Model depicting determinants that contribute to the risk of onset and maintenance of common chronic overlapping pain conditions. Maixner W et al. Overlapping Chronic Pain Conditions: Implications for Diagnosis and Classification. The Journal of Pain, 17(9) Supp 2 T93-T107

An article by Berk son in 1946 pointed out that sampling in particular ways can give misleading answers. It was thought that gall bladder disease could influence the risk of diabetes. People were having their gall bladder removed as an attempted treatment for diabetes; also, if you had more than one condition you were at increased likelihood of being admitted to hospital.

Berk son pointed out that if a study only includes patients in hospital, there seems to be an association – but a study of the wider population gave no association. This became known as Berk son bias; in causal analysis, it is an example of a collider bias. This is an important consideration in study design, and can be illustrated by using a toy example – see Figure 7.

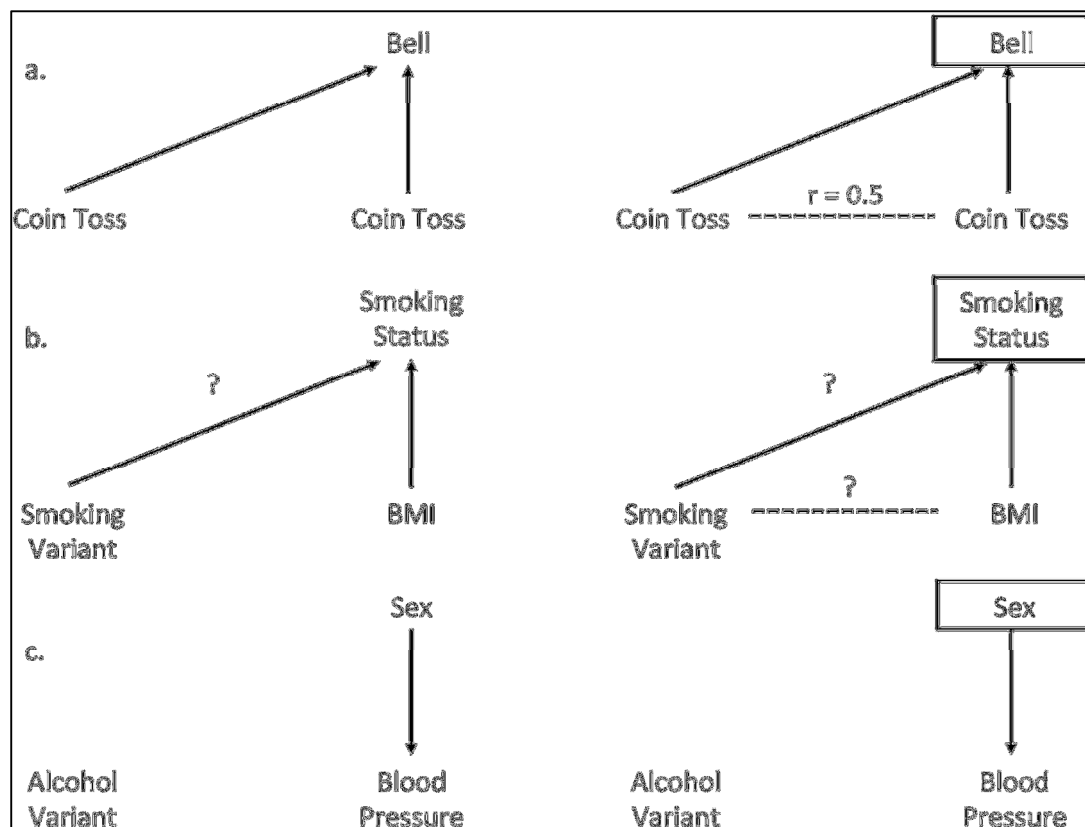


Figure 7: Illustration of collider bias. Panel A shows the basic premise of collider bias. In this example, a bell is sounded wherever either coin come up “heads.” The result of one coin toss is independent of the other. However, if we stratify on the bell ringing, seeing “heads” on both coins is not independent and a spurious correlation is induced. Panel B shows this with the example of stratifying on smoking status. If the variant used as an instrument for heaviness of smoking is also associated with smoking status (ie. ever-smoker versus never-smoker), and if BMI also influences smoking status, then there is a risk of collider bias if we stratify on smoking status. Panel C shows an example where stratification will not introduce collider bias, as sex is not an effect of either possession of a genetic variant that predicts alcohol consumption or of blood pressure. Source: Gage SH, Davey Smith G, Ware JJ, Flint J, Munafò MR (2016) G = E: What GWAS Can Tell Us about the environment. *PLoS Genet* 12(2): e1005765. doi:10.1371/journal.pgen.1005765

If sampling is related to highly proscriptive definitions, this can lead to automatic associations of factors amongst study participants that do not reflect what exists in the underlying population – or reflect meaningful or potentially causal associations, eg. gall bladder disease appearing to cause diabetes when it does not.

If representative samples with available data are recruited, the “proscriptively defined group” can still be investigated and if biases cause the associations this can be revealed. Even if proscriptive definitions are not biasing findings, they do not help – and indeed generally weaken – efforts to identify meaningful associations.

Prof Davey Smith concluded that using strict inclusion criteria is a lose-lose situation. An example highlighting this is a genome-wide association study into migraine (Atilla et al, 2013). As with CFS/ME, there were debates about what is or is not a migraine, whether tension headaches are a type of migraine, and whether the study should have been restricted to participants that have migraine with aura. If that restriction had been followed, very few findings would have emerged.

Having very broad categories for inclusion means more is revealed about the underlying liability, such as a shared biological basis between migraine without aura and coronary artery disease but a negative correlation genetically (Winsvold et al, 2015), and a positive genetic correlation between migraine without aura and ischaemic stroke (Malik et al, 2015). These correlations would not have been found if only migraine with aura had been studied.

Another example is the Avon Longitudinal Study of Parents and Children, involving a cohort of children with samples taken from cord blood when they were born, and followed up for 25 years. This was a very representative sample at initiation and the study examined whether these children continued to participate in the study.

It is now known that there are 108 genetic variants associated with schizophrenia risk, which are also present in the general population. A score can be calculated for how many variants an individual has. Very few people have zero and very few have 108, giving a score with a normal distribution. Looking at study participation as the child grows older revealed that those continuing to participate had lower scores for schizophrenia variants, whereas non-responders had higher scores.

The non-participation was not because they have developed schizophrenia (they were too young to do so) but it reflects underlying liabilities, like willingness to take part in studies. That can lead to serious biases in studies and associations seen in participants that are different to those in the general population.

Conclusions

Virtually all medical conditions are either directly part of a spectrum (like hypertension) or reflect a liability which lies along a spectrum. Subgroups within broad disease groups may reflect varying combinations of liability (“diathesis”) and specific exposures (“stress”).

Both are important. Selection of apparently highly specific case groups, even when intended to clarify the situation, can create biases. Attempts to identify and dissect such subgroups should be carried out at the analysis stage (with replication). Broad, representative criteria and recruitment strategies should be used for informative population-based studies.

Key point summary:

- Epigenetics is the study of the alteration in gene expression, which does not change the DNA sequence.
- Epigenome can give us information about: previous exposures (risk factors), predict future health (a diagnostic or prognostic aid) and can be used as a mediating mechanism linking risk factors with CFS/ME.
- For epigenetic studies, blood, saliva or other tissue samples are needed for DNA extraction.
- There are a number of technologies which can be used to study epigenetics, such as array based methods.
- A good bioinformatics infrastructure is essential in these studies to help integrate specific pathways or biological processes.

Epigenetics may be used as a molecular biomarker which may be useful in a population or patient based study of CFS/ME. Epigenetics is a relatively new area within the field of genetics; however, it specifically differs from genetics as it is the information that is overlaid on our DNA sequence. In simple terms, it signposts genes when to turn on or off, directs proteins where to dock with the genetic code, which parts to read, at what time and what to develop. Essentially it is the molecular mark laid on top of DNA.

In scientific terms, epigenetics describes a phenomenon in which genetically identical cells or organisms express their genomes' differently, causing variation in the cells and tissues they give rise to. Different epigenetics modifications lead to different gene patterns, which is the reason why some identical cells may become diseased.

Epigenetic marks determine tissue differentiation by directing gene expression. Epigenetics is the reason why our identical genome in every cell give rise to more than 200 different cell types and tissues due to the code being read in subtly different ways depending on the molecular code that overlays the genome.

The molecular signatures or markings that cause epigenetics result from chemical modifications. Of these chemical marks, methylation (one carbon and three hydrogens) is the most well-known and studied. The methyl groups attached to DNA at certain base sequences can then be measured very accurately.

From this we can calculate the percentage level of methylation. If heavily methylated, the gene is silenced or closed down; conversely if there is very little methylation occurring, the gene is in an open and readable state and is expressed.

The epigenetic markings are not fixed but instead influenced by environmental factors. Epigenetic variation has been associated with factors such as:

- diet
- diurnal/ seasonal correlations

- disease exposure
- toxic chemical
- drug abuse
- financial status
- exercise
- microbiome
- therapeutic drugs
- alternative medicine
- social interactions
- psychological state.

Further to the environment effects, epigenetic plasticity exists and is more pronounced at certain stages in the lifecourse. Epigenetic patterns measured in adulthood can tell us about factors we were exposed to *in vitro* and during childhood. *In vitro* and childhood are seen to have an important influence on epigenetics.

Additionally later in life we get less and less efficient at maintaining our epigenetic patterns, known as epigenetic drift; this in turn may contribute to age-related diseases. Epigenetic variation has been linked to a whole host of diseases such as cancer, brain disorders, chronic diseases and prenatal changes.

Prof Relton went on to explain that there are many issues and problems in establishing causality in epigenetic studies and these problems are very relevant to looking at something CFS/ME. For example, risk factors such as influenza exposure are linked to the onset of CFS/ME; however, it is hard to tell whether influenza might cause epigenetic change and then CFS/ME or whether any epigenetic changes are completely unrelated to the development of CFS/ME.

Prof Relton then went on to give a few examples of previous work. For example, the epigenome can be used as an exposure indicator, where DNA methylation signatures can distinguish between:

- current, ex-smokers and never smokers
- adolescents with transient exposure to cigarette smoking
- neonates exposed *in utero* to maternal cigarette smoking
- adults exposed to maternal cigarette smoke *in utero*.

This work shows that the epigenome is a really useful indicator of risk factors for disease and can capture a lot of useful information that goes far beyond a simple medical questionnaire (which is based solely on what a person can remember).

Another example is that this can be used to calculate epigenetic age. An epigenetic clock is a type of DNA clock based on measuring natural DNA methylation levels to estimate the biological age of tissue, cell type or organ. For a faster clock, you are older than your chronological age predicts, and this may be indicative of biological ageing; an older epigenetic age is associated with increased mortality. Results for epigenetic age predictions using current methods are roughly accurate to within two years.

Epigenetics can also be used as a predictive tool for early diagnosis or screening, such as in oropharyngeal cancer using saliva or peripheral blood DNA. The epigenome can also give a

clear indication of the response to treatment, in addition to serving as a clear biomarker to show the onset of disease.

Prof Relton then went on to show a few examples of published studies where epigenomics has been used as a diagnostic tool for brain tumour subtypes (Danielsson et al, 2015), as well as in studies of depression and changes in DNA methylation (Dempster et al, 2014). Further examples include how epigenomics can be used to show differences in pain phenotypes due to changes in DNA methylation (Sukenaga et al, 2016). In this study of 12 chronic pain patients, a correlation was found between DNA methylation levels and neuropathic pain symptoms, however it is not possible to conclude that this link is causal. Further to this, DNA methylation signatures have also been associated with inflammatory bowel disease, mood disorder and sleep disorders.

Prof Relton then went on to talk about the technology that is used in epigenetic studies; it largely follows on from the genome technology explosion in the last few decades used to study differences in genetic code. Typically used is an Infinium methylation EPIC bead chip for measurements. This chip-based, array-based technology measures 850,000 methylation sites across the genome. This technology is capable at generating massive data sets and very complex sets of information.

The last section of Prof Relton's talk looked at findings of the Avon Longitudinal Study of Parents and Children (ALSPAC). This study showed how epigenetics can be used to study exposure factors over long periods; findings include the following.

- Smoking during pregnancy is associated with changes in methylation, which persists sometimes for decades.
- Maternal obesity in pregnancy is associated with widespread variation in DNA methylation in children.
- Maternal B12 levels are associated with differences in childhood methylation and cognitive performance.
- Epigenetic age is a marker of childhood development.
- Type 2 diabetes is associated with methylation variation in adults.
- Children who develop conduct problems display differences in epigenetic patterns at birth.
- Assisted reproductive technologies are associated with methylation variation in childhood.

Routinely collected data for research
Prof David Ford, Swansea University

Prof Ford explained that he would be offering something different to previous speakers, in that he would be looking at how he and his team use data to examine health from a social perspective. Social circumstances are significant causes of ill health, and ill health has enormous social impact on patients and their families, he said. This is a societal problem that has an impact on us all.

In trying to understand this, Prof Ford will provide three linked case studies from his group's work to illustrate how routine data can support research into poor-understood conditions such as CFS/ME.

The first example described was Secure Anonymised Information Linkage Databank (SAIL). Set up in 2006, it captures data from the Welsh population, with around 20 billion recordings made to date. Originally it focused just on healthcare but is now a citizen-based repository that includes data from many sources including social care, housing, education, police and many others, all linked together to provide millions of anonymous individual 'stories' going back 20-30 years. Around 500 data sets are supplied every month, including general practice data from around 80% of GP surgeries in Wales.

The privacy of individuals whose data we keep is our first priority, said Prof Ford. The data never leaves SAIL and any researcher who uses it must access it via an online platform so the team can monitor how it is being used. No identities are contained in the data set, thanks to an automated process that keeps names and addresses separate from sensitive data.

Prof Ford then presented a case study of how the Swansea team operates a new Administrative Data Research Centre as part of a UK Network funded by the ESRC, which gives social and economic researchers access to a wide range of administrative data from central government departments such as social welfare, the Census, education, and many others.

He then moved onto to describe a programme of work that has been running for about five years, funded by the MS Society – the UK MS Register. By taking data from 25 clinical sites around the UK, and linking it to information provided by people living with MS directly, via a web portal, they have established a scalable register of people with MS, 15,000 to date, across the UK, regularly measuring their quality of life, functional ability and mood as well as many other aspects of their lives, condition and treatment. Those who share their data have given full consent to supply data regularly and routinely, building up a longitudinal resource – about 390,000 questionnaires have been completed so far.

"We also ask them to tell us a lot about their pre-illness history, life stories, social circumstances, employment, medications and treatments – some of which we get echoed in GP and hospital data, but some of it we don't, as there's lots of self-medication going on," commented Prof Ford. Data on fatigue, pain and cognition is also collected, and real-time collection of data on mood, and activity is about to start being collected using a patient portal smart phone app.

This sort of data is hugely valuable for anyone looking at a complex and disabling course of illness, and is supplemented by robust clinical data from a number of clinical sites across the UK.

Prof Ford ended his presentation by coming back to CFS/ME. “Until such time as there is a universal therapy for this very difficult condition, we should also spend time trying to understand how it’s presented and the affect it has on people’s lives,” he said. “I think the approaches described could really help illuminate CFS/ME, and I hope we will be able to help in the future.”

WORKSHOPS

Delegates were able to choose from three workshops at the conference.

Exploring the challenges of gaining CFS/ME funding for the range of science required ***Prof Paul Little, Southampton University***

This workshop was discussion-based with perspectives from a funder – Prof Tom Walley, National Institute for Health Research – and a person with CFS/ME, who first became ill in 1991.

“It was a glandular fever-type virus that rendered me bedridden for nine months,” she explained. “Not only was I confused by the illness but also confused by the lack of empathy, understanding, knowledge and facts. There seemed to be a perverse lack of ability or will to help. I was really shocked by the unwillingness of some medical professionals to believe this was a genuine medical condition, and the prevailing attitude at the time. This was not helped by the way CFS/ME was reported in the media, which trivialised the condition.

“When you think where we have come from, to where we are now, launching this amazing MEGA study which encompasses so many diverse disciplines, with so many of you ready to work in collaboration... it is beyond my wildest dreams. I am looking forward, with great expectations, to the results.”

Research with people with severe CFS/ME ***Victoria Strassheim, Newcastle University***

Victoria Strassheim is a chartered physiotherapist who has a background in neuro rehab. She has been working on a project to understand the clinical characteristics of severe CFS/ME and the relationship with cognitive and autonomic dysfunction,

Victoria led this workshop for researchers and people affected by CFS/ME to discuss challenges and possible solutions around including the most severely affected in CFS/ME research.

CFS/ME and POTS ***Dr Lesley Kavi, POTS UK and Prof Julia Newton, Newcastle University***

Dr Kavi gave an overview of Postural Tachycardia Syndrome, and her colleague, Nurse Practitioner Lorna Nicholson, described the results of the 2015 PoTS UK patient survey¹.

Prof Newton spoke about the current CRESTA service in Newcastle for CFS/ME and PoTS patients and the current status regarding research in the UK. The talks generated considerable discussion from the audience participants.

¹ http://www.potsuk.org/UserFiles/File/CFS_ME_research_collab_final.pdf

MRC-FUNDED PROJECT UPDATES

Biological Fingerprints of Fatigue in Primary Sjögren's Syndrome

Dr Nadia Howard-Tripp, NIHR Academic Clinical Fellow in Rheumatology, on behalf of Prof Fai Ng

Using a cohort of patients with Primary Sjögren's Syndrome, Prof Ng's team looked for unique patterns of biological changes that create "biological fingerprints." These can be found in the blood and may be relevant to CFS/ME.

Sjögren's, a chronic, inflammatory autoimmune disease, is systemic but its main symptoms include ocular dryness and profound fatigue. "Seventy percent of people with this illness report a disabling fatigue," explained Dr Howard Tripp, with up to two-thirds of patients fulfilling the criteria of CFS/ME.

With the emphasis on immunological pathways, the hunt was on for a biological profile, looking particularly at differential expression of certain genes between those with high fatigue (equal to or above 75 on a visual analogue scale) and those with low fatigue (equal to or below 25 on a visual analogue scale).

Using the UK Primary Sjögren's registry, which contains a biobank with serum samples and biological and clinical information, they looked at whole blood RNA in a relatively small sample. They found no differences between these two different fatigue states. But the comparison between the Sjögren's group and healthy controls was a different story, with 334 genes expressed differently.

They then took a look at type I Interferon gene signature as it is present in the majority of patients with Sjögren's and implicated in numerous immunological processes and disease. But fatigue levels and this particular gene signature were poorly correlated.

Moving on and hoping to find exactly which genes were relevant to fatigue, they used a statistical approach called Gene Set Enrichment Analysis which identifies clusters of genes that are functionally relevant to a particular disease.

"The aim was to find a fatigue related metabolic pathway," said Dr Howard-Tripp. Using software (Leading Edge) that picks out core genes that account for altered gene signalling, they found 19 pathways that were relevant. Interestingly, three of these had previously been implicated in CFS/ME: the Actin pathway, G-protein signalling and the Incretin pathway.

Using a machine learning approach, they were able to reliably sort between high and low fatigue groups. The results have been published in a recent paper.

Understanding the pathogenesis of autonomic dysfunction in CFS/ME and its relationship with cognitive impairment

Professor Julia Newton, Director of Newcastle Academic Health Partners and Clinical Professor of Ageing and Medicine, Newcastle University

Prof Newton gave an update of Newcastle's autonomic projects. First was a case control study designed to better understand the role that autonomic dysfunction has to play in the pathophysiology of CFS.

Initial investigations looked at whether autonomic dysfunction was a primary abnormality in brain autonomic centres or due to abnormalities in the HPA (hypothalamic pituitary adrenal) axis. They also considered whether it could be a problem downstream of the autonomic nervous system that is secondary to hypovolaemia. This in turn might result in compensatory autonomic abnormalities.

Secondly, they explored autonomic dysfunction and cognitive impairment in CFS/ME. The researchers wondered if underlying central processes cause damage to both autonomic and cognitive brain centres, or whether cognitive impairment is secondary to peripheral hypotension arising from autonomic dysfunction. They also looked into whether the potential reduced cerebral perfusion had caused cerebral damage.

A wide range of investigations was carried out in a cohort of over fifty patients with CFS/ME. Funding allowed three MRI scans per participant of brain, heart and liver, and a number of nucleotide investigations including plasma volume, red cell volume and MIBG.

"We also performed a full gamut of autonomic tests, a dexamethasone suppression test as a measure of HPA axis function and a full battery of neuropsychometric assessment all in the same participants," said Prof Newton. "The considerable advantages of that mean we have the ability to look at a whole systems approach to CFS/ME."

Though the ongoing analysis is taking a huge amount of time due to lack of continued funding, the team have developed a number of new methodologies around liver and brain MRI so will be publishing methodological papers.

Further outcomes of the project were wide ranging and abundant. The PhD muscle function work has gone on to receive funding from the MRC Confidence and Concept scheme for Audrey Brown, lead author on the PLOS paper on muscle glucose uptake (Abnormalities of AMPK Activation and Glucose Uptake in Cultured Skeletal Muscle Cells from Individuals with CFS. Brown, AE et al. *PLoS One*, 2015). Prof Newton expressed her delight in recently being invited for full application from MRC to develop this work.

Currently three additional papers are being written. Though the HPA axis and autonomic dysfunction study show no correlation, the cardiac data MIBGs (a type of scan) have been very positive, as has research looking at plasma volume and red cell mass. Cardiac work suggests that patients with CFSME have small hearts and small cardiac volumes, something that both the Newcastle team and others have shown before using different methodologies.

“People with CFS/ME have low plasma volumes, and the lower your plasma volume is the more fatigued you are,” explained Prof Newton. “This is the basis of a current study where we are looking at expanding plasma volume with oral fluid and intravenous fluid.”

Funded by Action for M.E., this proof of concept study will underpin the MRC DPFS (Developmental Pathway Funding Scheme) application to be submitted in the new year. Some of the cardiac data already been published in *Open Heart* (Reduced cardiac volumes in CFS/ME associate with plasma volume but not length of disease a cohort study. Newton et al. *Open Heart*, 2016) has been downloaded more than 10,000 times.

“One of the big things we learnt from this project is that doing research with this population is really doable and that people really want to participate,” said Prof Newton. The key to success is the recruitment team’s exceptionally good relationships with the local patient support groups and the patients themselves. Despite it being really difficult for patients to undertake the assessments, every one of them completed all of the tests. And she confirmed the team had utilised every single bit of information the participants had given.

Academic papers and grants were the obvious tangible outputs of her research group, but gains from the MRC and charity funding went much further. “It’s brought us credibility in Newcastle,” she said. “We now have a recognised Newcastle University fatigue research centre, which cross-faculty. That gives us a small amount of funding to allow people to come to meetings and to facilitate Fellows and researchers to come and work with us.”

This in turn has produced a large number of national and international collaborations, including Faisal Kahn in Dundee, the mitochondria group at Oxford University and across the globe with researchers in Poland, Australia and the US.

“It’s great talking to people about the science we’ve done and helping facilitate their science. That can only be good for the field,” she concluded.

Answering a question from a delegate, Prof Newton explained why she thought the HPA function test showed no difference to controls. “We’ve now looked at HPA function in two different ways with the dexamethasone suppression test. We’ve looked at not only cortisol levels but a range of different cytokines to look at whether there are changes when we depress HPA function and we have not been able to find any changes that are significant in CFS/ME compared to controls.

“We’ve also taken a cohort of patients where we’ve taken salivary cortisol over 24 hours and looked at circadian rhythms and again not been able to find abnormalities in CFS/ME versus controls,” she said. The main reason for the difference could be that researchers screened out rigorously for depression.” It maybe that depression as a cofounder is the complicating factor.”

Persistent fatigue induced by interferon alpha: a new immunological model for CFS/ME

Alice Russell, King's College London, on behalf of Prof Carmine Pariante

Prof Pariante's team looked at the pro-inflammatory cytokine interferon alpha in relation to persistent fatigue. A natural part of the immune response, interferon alpha is also created synthetically for therapeutic use in Hepatitis C. Patients inject it for six to 12 months to boost their natural immune response and inhibit replication of the virus. It does however have a wide range of side effects.

According to a survey conducted by the Hep C Trust in 2010, 60% of patients report problems with fatigue 6-12 months after the treatment has stopped and around 40% are still fatigued after a year post-treatment.

"It's not just fatigue, it's a range of symptoms that are very similar to what you see in CFS/ME," explained Miss Russell. "And that's why we think interferon-alpha induced persistent fatigue is an interesting proxy model."

The first part of the project was a prospective cohort study, following patients from four sites across London who were receiving Interferon Alpha for Hep C. Fifty eight patients completed the treatment and were seen a minimum of 6 times including before during and after treatment.

The second part was a case control cross sectional study with CFS/ME patients. Fifty two CFS/ME patients from two sites in London and 58 healthy controls attended for one of visit where a raft of measures were taken. These were the same as those taken from the Hep C patients over time.

Initially looking at Hep C patients, they compared those whose fatigue had persisted post-treatment, to those whose fatigue had resolved, to try to understand whether there were any differences between the two groups even before treatment, which might explain the different outcomes later on.

Predominantly male, which Miss Russell acknowledged as being very different from standard CFS/ME cohorts, they had similar socio demographic data, Hepatitis C genotype and level of virus and similar damage to their liver, assessed by a fibroscan.

Results so far have proven interesting. Illness severity (viral load) measured before treatment made no difference to post-treatment fatigue levels. When the treatment worked more quickly (the virus was cleared after four weeks) there was more likely to be a resolution of fatigue. However, whether the treatment worked, and the virus was still cleared six-months post-treatment, did not make a difference. This was contrary to the expectations of the liver consultants, who thought that any fatigue would be due to a persistent Hepatitis C infection.

"That's important because what's starting to come out is that this early analysis is that this early response to the trigger might be the important thing in this group," explained Miss Russell.

The research team then looked at cytokine levels in the blood in a subset of patients. While levels of IL-10, IL-17A and IL-6 were increased in response to Interferon-alpha in all patients, they were significantly higher in those patients whose fatigue persisted post-treatment, suggesting a greater inflammatory response to the trigger in these patients. The increases in these cytokines in the first four weeks of exposure seemed most important.

Interestingly, this was despite those particular cytokines returning to baseline levels once treatment was completed. IL7 was markedly different however, in that it was higher six months after treatment in persistent fatigued patients compared to the resolved fatigue group. More results will be available soon. Miss Russell reiterated Prof Newton's experience, saying that the level of enthusiasm of patients wishing to participate was overwhelming.

Modulation of Aberrant Mitochondrial Function and Cytokine Production in Skeletal Muscle of Patients with CFS/ME

Prof Anne McArdle, Institute of Ageing and Chronic Disease and MRC Arthritis Research UK Centre for Integrated Research into Musculoskeletal Ageing, University of Liverpool

Prof McArdle's research suggests that there are strong similarities between muscle dysfunction in CFS/ME and that seen in old age.

When you're young and healthy your muscles can adapt, and with training they get bigger and more efficient, explained Prof McArdle. But one of the by-products of this energy production are free radicals, in particular reactive oxygen species (ROS).

It was previously thought that these were produced predominantly by the mitochondria and, though important in various cellular processes, they are implicated in inflammation and damage to DNA and tissue. But work by Liverpool's Prof Malcolm Jackson has shown that far from being the baddies of energy production, free radicals are crucial for adaptation in healthy muscle. And interestingly, it's the NADPH oxido reductase complexes in the plasma membrane, rather than the mitochondria in this instance, that is producing it.

The story gets even more intriguing when you look at the difference between old and young muscle. "You can take a muscle and hypertrophy it, you can make it bigger," said Prof McArdle, "But old muscles can't do this." And the Liverpool team think they know why.

By isolating individual muscle cells, they found a normal increase in ROS in young healthy muscle, but in old muscles these are already elevated. Old muscles cannot produce further amounts of ROS, so signalling capacity is lost and the muscle is no longer able to adapt to exercise. Unfortunately, that's not all. Certain chaperone molecules such as heat shock proteins that assist in adaptation are reduced while inflammatory and immune markers are increased. "What you have is a chronic activation of NFkappa B in those muscles," explained Prof McArdle, "and they are actually churning out cytokines."

It's becoming increasingly clear that it is the muscle environment that is key to dysfunction. Prof McArdle's old age research shows altered ROS generation, hydrogen peroxide generation by mitochondria and a chronic activation of NFkappa beta (a key regulator of the immune system). In aged mice, this causes an increase in a whole panel of cytokines within the muscle itself.

More evidence that the muscle environment influences force generation, comes from a study which involved transplanted young muscle to old mice and via versa. "Take a young muscle, put it in an old mouse, it behaves like an old muscle and we think that inflammatory environment is playing a major role there," she explained.

So how is this relevant to CFS/ME? Prof McArdle has just finished an MRC-funded study and is still working on the data but the results look promising. In a cohort of hundred patients and a hundred healthy volunteers' cytokine panels were analysed.

"The reason we did this is particularly important," explained Prof McArdle. "We know that muscle weakness and exercise intolerance is a secondary consequence to many inflammatory disorders. Cytokines such as TNF α play a major role in modulating the function

of muscle and we now know that muscle can be a major endocrine organ producing a vast array of cytokines.”

Muscle function and muscle biopsy studies were done in a subgroup of 17 patients. Though mitochondrial membrane potential was relatively normal, said Prof McArdle, when they looked at mitochondrial hydrogen peroxide production it was “tantalisingly elevated in all respiration states of the mitochondria.” There were also significant elevations in levels of cytokines in the muscle. “And we see tantalising increases in serum cytokine levels in our patient groups,” she added.

Cytokines have a big impact on function. “If we treat mice with TNF α , the force generation is significantly down,” said Prof McArdle. But the Liverpool team are already working on a treatment using HSP10 (heat shock protein 10) that appears to block the increase in TNF α , which in turn blocks production of hydrogen peroxide and cytokines.

They are continuing their data analysis, trying to determine subgroups of chronic fatigue patients with altered cytokine profiles. Current projects also aim to understand local effects of muscle produced cytokines, muscle innervation and the change in muscle fibre environment which Prof McArdle thinks might be altered in chronic fatigue patients. But it’s their work on old muscles that might provide some interesting therapeutic interventions. The Liverpool team are currently developing and testing supplements for their old age patients and these could potentially be used to help people with CFS/ME.

Slow wave sleep and daytime functioning in CFS/ME

Dr Sue Wilson, Hon Senior Research Fellow, Centre for Neuropsychopharmacology, Imperial College London

Sleep problems are very common in CFS/ME, with many reporting disturbed sleep, hypersomnia and waking unrefreshed. Patients generally feel that if they have a better night, they go on to have a better day, explained Dr Wilson. “There is some quite good evidence that the structure of sleep, drilling down into the micro-architecture of sleep, shows deficiencies in slow wave sleep in CFS/ME.”

An intense commitment was required of the 10 participants who undertook the study. Patients stayed in the research unit for five days on two separate occasions with respiratory variables being measured every night. Sodium oxybate or placebo (double blind, randomised crossover protocol) was given on four nights. “We pharmacologically enhanced the slow wave activity in slow wave sleep just to see if makes any difference to functioning during the day,” said Dr Wilson.

On the first and fourth nights, sleep was recorded with polysomnography along with an electroencephalogram (EEG), muscle activity, eye and leg movements. Day time functioning was assessed on day two and day five using a variety of tests including multiple sleep latency tests (4 nap opportunities) to measure objective sleepiness, grip strength and fatigue, cognitive testing using computerised and pencil and paper task and several visual analogue scale including of mood, exhaustion and irritability.

With a Pittsburgh sleep questionnaire averaging over five, participants sleep was subjectively poor. But unlike reports from other groups, a low Epworth score showed they weren’t actually sleepy during the day time.

The intervention drug, meanwhile, threw up unexpected results. “We did manage to increase slow wave sleep on both the first night and night four,” explained Dr Wilson. “We’ve started to do the spectral analysis on very slow wave sleep and it looks like that was increased too. But what we also seem to have done is significantly increase waking during the second half of the night.”

Morning sleep satisfaction, objective sleepiness and tests of executive function showed no significant difference between drug and placebo groups.

One of the most interesting points to come out of the study was that many CFS/ME patients have comorbid sleep disorders.

“A large number of patients were excluded for sleep disordered breathing at the screening stage [which involved an overnight EEG], many more than we would have expected,” said Dr Wilson. “There were other sleep disorders including restless legs syndrome and these figures were very high.”

The research team want to get together with other CFS/ME scientists to discuss the implications and treatment options for this novel finding.

NEW RESEARCH

Mapping global research funding over the last 10 years: a UK CFS/ME Research Collaborative-sponsored report *Sonya Chowdhury, Chief Executive, Action for M.E.*

Key point summary:

- The ÜberResearch Dimensions database of over 200 of the world's most influential research funders was queried.
- Over the ten years covered by the report there were 99 grants awarded globally, with a total of around £49m.
- CFS/ME receives only 0.02% of the overall total of research funding
- Considering the burden of CFS/ME and the number of people affected, research receives disproportionately low funding compared with that for other neurological illnesses.
- The CMRC will offer support to researchers to develop better quality funding applications and study design.

Sonya began the New Research plenary session of the conference by presenting the findings of a report² into CFS/ME research funding over the last ten years. The report gives hard evidence of the chronic lack of research funding for CFS/ME from the major funding agencies. While this is already widely acknowledged, the CMRC will use this as evidence to present to the MRC, NIHR, Wellcome and other research funding. The method for the report involved working with a company called ÜberResearch, using their Dimensions database of over 200 of the world's most influential research funders. There are gaps in the data in terms of the database, which does not include research funded by charities, but it nevertheless gives a snapshot of the major research funding over the last 10 years. Over the ten years covered by the report, although there are some omissions, there were 99 grants awarded, with a total of around £49m. A breakdown by country is shown in figure 1.

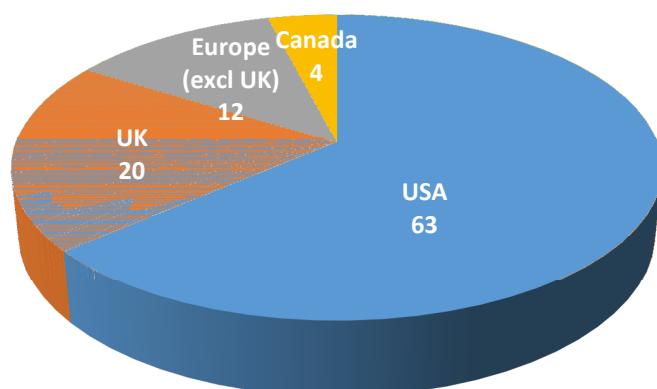


Figure 4: CFS/ME research grants per country over the last 10 years

² www.actionforme.org.uk/uploads/pdfs/mecfs-research-funding-report-2016.pdf

Figure 2 shows the number of active and starting projects each year, excluding the UK, for the ten years up to 31st December, 2015. The number of new projects varies from year to year but on average there are six new projects funded per year excluding the UK, and this is low compared with funding for comparable illnesses. Most of the funding awards have come from the NIH in the US, and since the US Institute of Medicine's 2015 report there has been a review of funding.

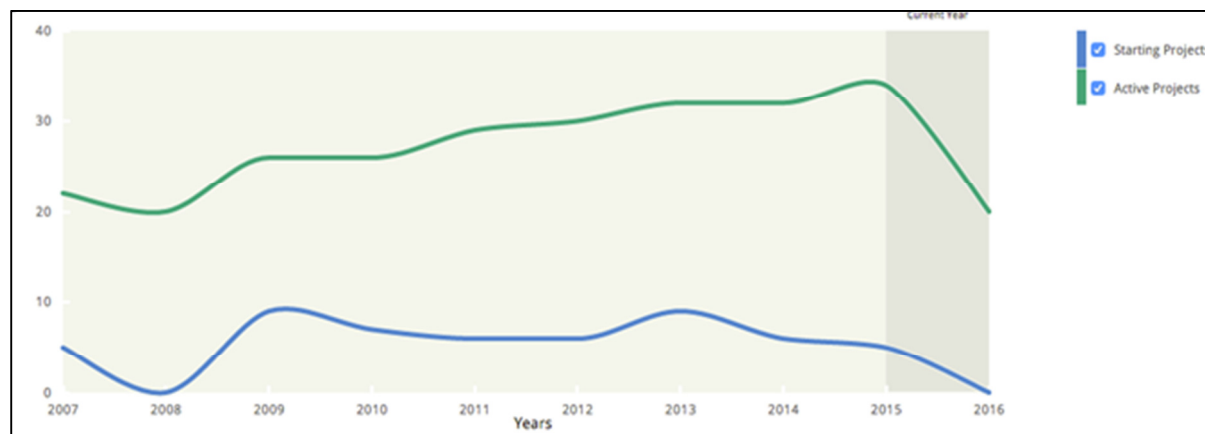


Figure 5: Global CFS/ME starting and active grants (excluding the UK) since 2007

Figure 3 shows that for UK CFS/ME research funding since 2007 there were on average only two grants awarded per year. There is a spike due to the MRC highlight notice in 2012. A breakdown of grants awarded per institution and by PI recipient can be found in the full report. Of the 20 grants in the UK, two fifths went to the same two researchers.

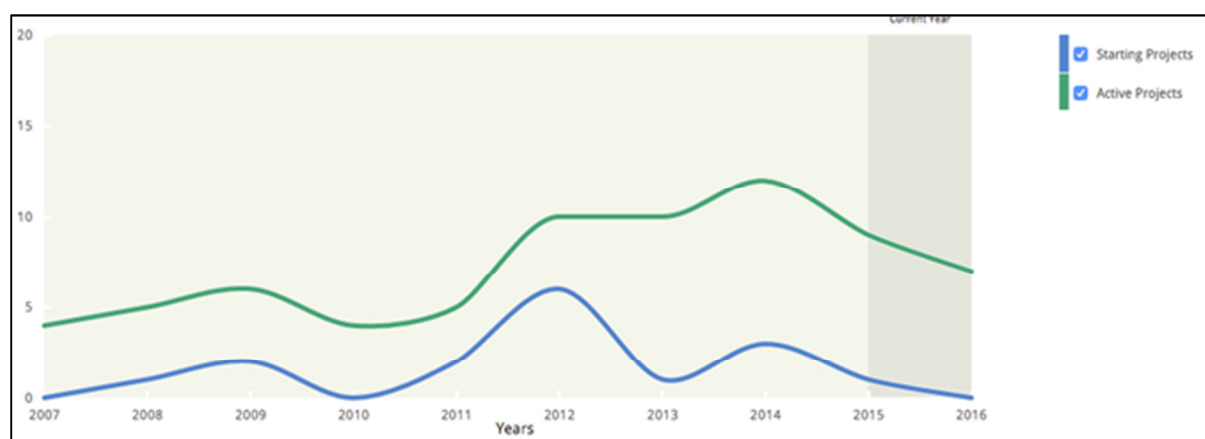


Figure 6: Grants for CFS/ME research from funding agencies in the UK since 2007

There has been no funding from other UK and European funders, such as the European Biotechnology and Biological Sciences Research Council, or the European Research Council for whatever reason.

Of the 20 grants funded in the UK in the 10-year period, the breakdown by funder was:

- 12 from the MRC (due to the highlight notice)
- 6 from the National Institute of Health Research (NIHR)
- 1 from the Chief Scientist Office in Scotland
- 1 from Wellcome.

Funding was then compared with that given for other neurological conditions, as shown in figure 4. In the ten years to 31 December 2015 there were 34 international active grants in the Dimensions database for CFS/ME, with a total value of around £17m. To put this into context, the MRC funded around 2,000 active grants and Wellcome around 200 active grants at that point in time.

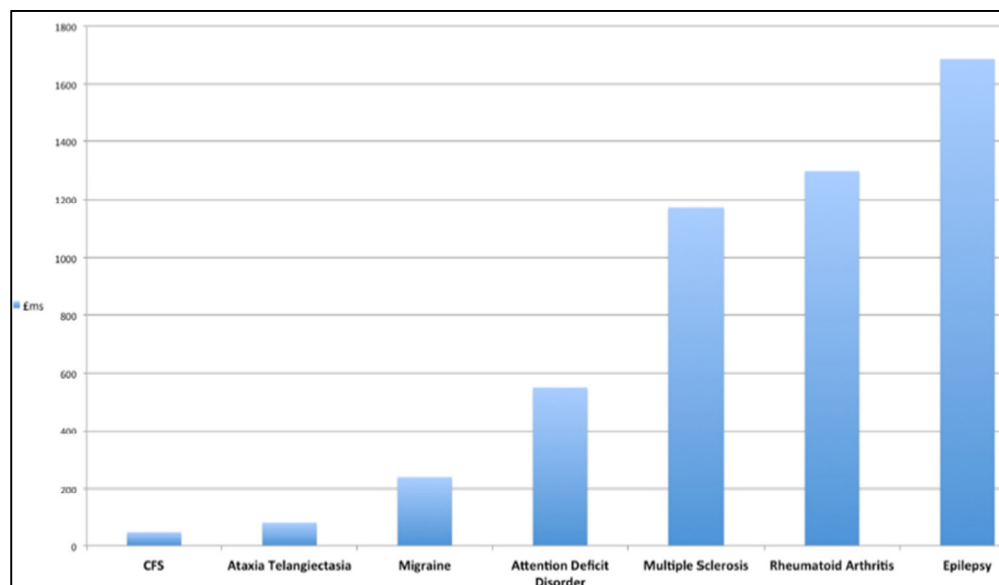


Figure 7: CFS/ME funding compared with other neurological disorders (worldwide funding)

As can be seen in the full report, there is disproportionate funding. For example, ataxia telangiectasia affects 1,200 people in the UK, but gets twice as much funding as CFS/ME which affects around 250,000. Multiple sclerosis affects around 100,000 but receives about twenty times the funding.

Each of these illnesses deserves funding and these comparisons are purely made to demonstrate the need for fairness in funding research to improve the lives of people with CFS/ME. It is not reasonable that this illness receives only 0.02% of the overall total of research funding.

The scale and the impact of CFS/ME is significant in terms of the cost of disease burden but research funding has been low and patchy. More skills and expertise are needed in research applications and design of studies.

The CMRC has been in consultation with the MRC and other UK funding agencies to increase investment. The MRC are going to formally review the report and their highlight notice, which is a major first step. The CMRC will be writing to each of the funding organisations to encourage them to work with us to increase research funding for CFS/ME.

A critical analysis of the categories of research funding will be carried out in order to identify under-researched areas of CFS/ME research for future investment.

The CMRC will be working with researchers to improve the success rate of applications for funding. The CMRC will offer advice to researchers in the early stages of their career to enable them to submit high quality applications to the mainstream research funders.

There has been an incredible lack of research and focus which needs addressing, so the CMRC encourages researchers to work with them and give their feedback, as the spotlight on funding will be ongoing.

Sonya ended her talk by showing a photo collage of patients affected by CFS/ME from the Millions Missing campaign, to remind delegates that the overall aim of research is to improve the lives of people affected by this illness.

Enhancing research funding applications into CFS/ME: part one

Dr Lindsay Keir, Senior Portfolio Developer, Wellcome

Dr Keir explained that she was going to present on the funding opportunities presented by Wellcome, formally the Wellcome Trust.

Wellcome is a charity but does not source funds from the public or donations, but instead funds research through a trust. Its remit is to improve health for everyone by helping great ideas thrive.

“To do this, we have four main aims,” explained Dr Keir. “These are to understand health and disease, to improve health, to engage with the public – including patient groups – and to influence policy.”

Many teams are involved in this work, including for example the Innovations Team, which works globally with researchers and organisations to transform ideas, discoveries and inventions into treatments and products; the Culture and Society Division funds researchers looking at the humanities and social sciences.

Dr Keir explained that her area of work was broadly improving healthcare via various funding streams, including molecular sciences and all the way through to population-based studies. Her team was established this year to particularly focus on clinical research, led by Sarah Marshall, a clinical immunologist by trade.

Wellcome supports research across the career structure, with different schemes available, including:

- an undergraduate scheme for basic and clinical researchers that includes a “biomedical vacation” scholarship to enable them to get laboratory experience
- postgraduate schemes that help fund PhDs across the UK
- Sir Henry Wellcome postdoctoral fellowships, which allow researchers to travel between labs and take ownership of their career direction
- clinical career development fellowships, which can run for up to eight years over two flexible stages
- Sir Henry Dale fellowships, which support the best researchers to progress towards independence
- re-entry fellowships, for those who have had to take time out of research for any reason.

Dr Keir then explained more about each of Wellcome’s specific funding streams for established researchers.

Seed Awards in Science help predominantly early independent researchers develop novel ideas that will go on to form part of larger grant applications to the Wellcome Trust or elsewhere. Awards can be up to £100,000 over two years and applications are considered three times a year. You must already have a salary in place for the duration of the award, so it’s really targeted at someone already in post for example a newly appointed lecturer or someone is more established but who wants to move into a new area. Applicants must have very clear follow on plans.

Larger awards aimed at funding established scientists include Investigator Awards in Science, which offer flexible funding support to researchers at all career stages working on important questions of relevance to our scientific remit, and can be anywhere from £300,000 up to £3million over seven years, although most people apply for 5 years of funding. You need to show that you have a strong track record relative to your career stage, and an over-arching research vision for the whole project. Applications are considered three times a year, and joint applications from researchers working closely together are being received more frequently.

Senior Research Fellowships are designed to support the best independent researchers asking important questions in their field. Offering up to £1.5million over five years, they can be renewed after that period. Around seven to 12 years' research experience at postdoctoral level is required, and an international track record, with sponsorship from your head of department.

Dr Keir stressed that the Wellcome team is very happy to give advice and guidance on individual applications, and that anyone interested should get in touch.

She then finished by talking about some of the newer awards that are available to researchers, including the Collaborative Award. The aim of this is to promote development of new ideas that speeds up the pace of discovery. "We fund teams of three to seven researchers, consisting of independent research groups, to work together on the most important scientific problems that can only be solved through collaborative efforts," she explained. These are large awards of £4million over five years, and eligibility includes having a "multi-disciplinary dream team."

How are these applications assessed? Dr Keir was keen to de-mystify this process.

She reiterated that some of the awards require preliminary application. This goes through an eligibility check, and a proportion will then be invited for full application. Expert review groups, made up of specialists in the field, will assess applications, around 40% of which will be successful and progress to the next stage.

These will be sent for sub-specialist peer-review, and then onto an interview panel, made up of people working across all six funding streams. "Your application will be seen by all these different audience, so you must pitch it correctly," advised Dr Keir. Overall, around 15-20% of all applications received are ultimately successful.

The final award outlined by Dr Keir was the Biomedical Resource and Technology Development Grant, aimed at researchers who want to establish or maintain resources or technology for the benefit of the wider scientific community, eg. a biobank. This award of up to £1.5m over five years is only considered once a year.

For more information, please visit www.wellcome.ac.uk where each award page has a contact lead.

Enhancing research funding applications into CFS/ME

Dr Neha Issar-Brown, Programme Manager for Population Sciences and Public Health, Medical Research Council

Key point summary:

In order to increase chances of success, particularly for early career researchers:

- Plan well: allow plenty of time, choose funder, scheme and partners/collaborators carefully. Interdisciplinary expertise can be particularly valuable in this field - to bring in new ideas / methodology;
- Present clear objectives: create specific aims, based on clear hypothesis and include well-defined criteria to quantify success. focus on a specific gap in knowledge in the CFS/ME field, and be ambitious but with realistic goals;
- Justify methodology: ensure that the study design has a sufficient level of detail, has the right numbers, statistical power and covers all bases, with the limitations of the design stated upfront;
- Seek advice early: get proposal reviewed internally first, consult experts (including those on UK CMRC) for guidance and comments.

The MRC has a cross-Board highlight notice in CFS/ME³. Highlight notices are used to alert researchers to areas of biomedical science that are currently a high priority for the MRC. They do not have a separate budget and applications against highlight notices are considered in competition with other applications received; however, strategic relevance to the highlight is one of the criteria considered during the review process.

While the MRC Population & Systems Medicine Board hosts the CFS/ME highlight notice, applications can be submitted to any of the four relevant science Boards⁴ through the many response mode funding schemes.⁵ All science Boards three times a year. Of note, is the New Investigator Research Grant scheme⁶ which is aimed at early career researchers who are capable of becoming independent Principal Investigators and are now ready to take the next step towards that goal.

The MRC also has some overarching panels that go across the following four science areas, which may be of interest to all CFS/ME researchers.

- Training and Careers: offers flexible support for people at key stages in their careers for early-career researchers. This information is available in an interactive career framework⁷ which gives information on possible options for both careers and funding in biomedical research within academia and/or industry.

³ <https://www.mrc.ac.uk/funding/how-we-fund-research/highlight-notices/cfs-me-highlight-notice/>

⁴ <https://www.mrc.ac.uk/funding/science-areas/>

⁵ <https://www.mrc.ac.uk/funding/how-we-fund-research/>

⁶ <https://www.mrc.ac.uk/funding/how-we-fund-research/new-investigator-research-grant/>

⁷ <https://www.mrc.ac.uk/skills-careers/interactive-career-framework/>

- The Methodology Research Panel: Methodology research, from an MRC perspective, is the study of how best to design, conduct, analyse and evaluate medical and health research and underpins all of our science areas. However, the Methodology Research Programme (MRP)⁸ is central to supporting the development of new methodologies in health research and the MRC leads this partnership with the National Institute for Health Research. The MRP Panel meets twice per year to review grant applications.
- The Translation Research Group: supports translational research across all of MRC's funding Boards and Panels, through partnerships with other funders and major Higher Education Institutions and through a range dedicated funding schemes.⁹

Why do applications in the field of CFS/ME fail?

Delegates suggested that applications fail because of the following reasons:

- studies are too small in design
- there is too much negativity in the field about harassment, preventing researchers to enter or continue working in this field
- prejudices of peer-reviewers.

Dr Issar-Brown explained that peer-review is the cornerstone of the MRC and that a very transparent, two-stage peer-review process is followed. This is presented very clearly and succinctly in an animation¹⁰ on the MRC's website, which she urged members to watch.

The MRC has a clear policy and process in place for selecting peer-reviewers and applies it consistently to all areas of research, as the integrity of peer-review is of paramount importance. This means that any personal interests of a reviewer must never influence, or be seen to influence, the outcome. As such, while we welcome peer-review suggestions from applicants, we source other relevant experts and ensure that all potential conflicts are carefully considered. More details on what MRC considers a conflict can be found on the MRC's website.

However, Dr Issar-Brown explained that what was perhaps more concerning was that often many researchers decline to be reviewers when approached, and thus finding appropriate reviewers in a timely manner becomes very challenging. This experience was echoed by the charities that fund research.

Dr Issar-Brown mentioned that all researchers supported by the MRC are expected to participate in peer review but urged other researchers in the field to make themselves available for peer-reviewing. In order to ensure robust and fair peer-review, she suggested that senior researchers in the field should ensure that less experienced staff receive appropriate training. The MRC also published guidance¹¹ on its website to this effect.

⁸ <https://www.mrc.ac.uk/research/initiatives/methodology/>

⁹ <https://www.mrc.ac.uk/about/our-structure/strategy-board-overview-groups/translational-research-group/>

¹⁰ https://www.youtube.com/watch?v=_DErve4a0IA

¹¹ <https://www.mrc.ac.uk/documents/pdf/reviewers-handbook/>

Making a successful application

There are several aspects to writing a successful grant proposal. The key aspects are highlighted below but this is not an exhaustive list.

The hypothesis: The study should have a long-term plan or global objective. What are you doing and why are you doing it? Provide a clear rationale. Feel free to show a little ambition and take on a problem – but make sure you can explain why and then convince us you’ve got a fair chance of doing it.

The focus: Focus on the knowledge gap(s) that needs addressing and show the uniqueness of your approach. Where possible, see how it aligns with a particular funders’ research priority or initiative. For example, finding sub-phenotypes within the disease population could be a priority for CFS/ME research and a proposal focussing on this may align with one of the MRC’s Stratified Medicine calls.

To this end, the CMRC’s funding report (see p 47) has already highlighted several knowledge gaps in the field and, particularly, the need for further, multifaceted research to identify the underlying mechanism and biomarkers. This should provide a good starting point for those within the field but also outside of it. Finally, while there are many unanswered questions, it is crucial to focus on some tangible ones.

The impact: This is basically answering the “So what?” question. Explain the intended consequences of your work. Who could benefit in the long term? How can you increase the chances of reaching those beneficiaries? Even if your proposal doesn’t directly address economic or societal impact you should be able to explain the pathway that links your work to improving human health. Academic and scientific impacts are equally important to consider.

For example, in the case of CFS/ME, the large societal and economic need has already been established however, questions such as the knowledge gap the research in question will fill (see above), how it will progress the methodology or the science and/or how translate to a therapeutic target or treatment etc. also need to be elucidated.

The method – The methods used should prove or disprove the hypothesis conclusively, and have the right numbers and statistical power. Use the right tools in the right way. Whether the proposal involves animals or human subjects, it is crucial that the statistical analysis is thoroughly detailed. This is particularly important in the case of CFS/ME research as small numbers (of patient cohorts/samples) with high heterogeneity can lead to misleading or inconclusive results and this is one of the key factors the applications fail at peer-review stage. Finally, if there are known limitations or risks, acknowledge and, where possible, address them and/or present appropriate mitigation strategies (Have a backup plan!).

Personnel and collaboration – *Who* proposes to carry out the study can be as important as *what* is being proposed. In this field where there are many early-career researchers, they may rely on more established researchers for mentorship and support. Thus, it is important to find the right people to collaborate with, both within CFS/ME research, as well as, outside of the field if it helps you answer the key objectives.

To this end, the CMRC can be used to internally review the application, drawing on the experience of other members. Members/ experts may also be able to point towards potential collaborators. Also ensure that someone from outside the field can read and understand the application, as it will need to convince a diverse MRC Research board with expert from different fields.

Dr Issar-Brown finished her presentation by commenting that, as discussed, statistical power and sample size are crucial to progressing the research agenda in this field. As such, population based investigations such as the proposed MEGA study offer a valuable opportunity to generate large volumes of useful data, which can be mined and exploited by the larger research community to understand the underlying mechanisms of disease.

Finally, she stressed that while the community's concerns about lack of investment in this field – as compared to other areas / diseases – are understandable, CFS/ME researchers need to be “in it to win it” and work with the funding agencies to ensure that sufficient high-quality applications are submitted to funders, to increase the chances of success and to advance research in this field.

The Brain in Pain studies: central sensitisation in CFS/ME and fibromyalgia
Dr Julius Bourke, Queen Mary's University, London

As part of this presentation, Dr Bourke shared raw data and has asked that it not be presented here, as publication in a peer-reviewed journal is being sought.

By way of general overview, the Brain in Pain studies are comparative studies of central opioidergic and dopaminergic neurotransmission in cases of fibromyalgia and CFS/ME that demonstrate central sensitisation and healthy controls that do not.

The principle of central sensitisation is derived from pain medicine and essentially infers a state of central nervous system hypersensitivity. This in turn results in unfiltered information being transferred up to the brain and all stimuli being treated the same – as a threat, as pain and with the need for preferential processing. Pain is a case in point but the effect is multimodal. Normal pain processing is shown in figure 1, and the effects of central sensitisation are shown in figure 2.

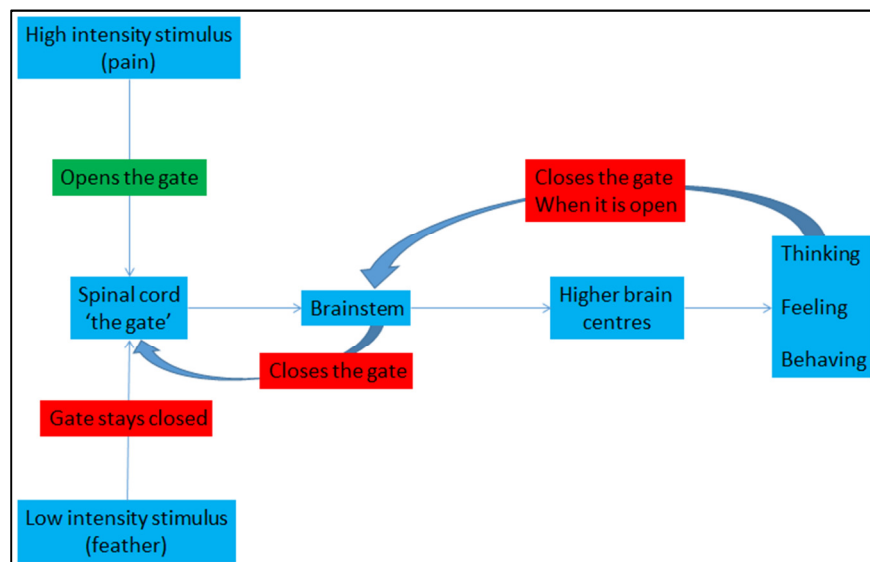


Figure 1: Normal pain processing

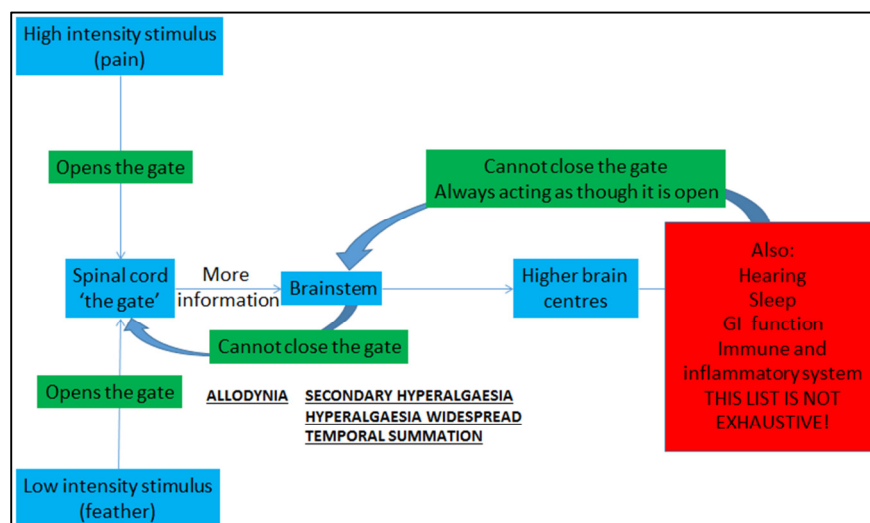


Figure 2: Pain processing in central sensitisation

Central sensitisation is found in numerous chronic pain disorders and is thought to play a role in CFS/ME, fibromyalgia, and other related disorders such as irritable bowel syndrome (IBS),

Pain thresholds have been demonstrated as being lower in these conditions and temporal summation or “wind up” has been reported. However, central sensitisation and temporal summation are not equivalent and low pain thresholds alone are not sufficient to demonstrate this altered state. CFS/ME and fibromyalgia are heterogeneous conditions but both involve chronic pain. Central sensitisation may help introduce greater homogeneity in research samples.

Although central sensitisation is thought to be a neurophysiological explanation of chronic pain, it may also explain the following:

- Heritability
- Susceptibility to developing CFS/ME and fibromyalgia
- cognitive and emotional dysregulation
- comorbidity of similar disorders (e.g. IBS)
- sleep dysregulation
- neuroendocrine dysfunction
- autonomic dysregulation
- sensitivity to noise, temperature, environment – social and occupational
- immune system dysfunction.

A better understanding of the central chemical processing underlying this hypersensitive state may lead to an enhanced understanding of conditions such as CFS/ME and may provide a target for future pharmacotherapy.

Voxel-based morphometry shows reductions in brainstem white matter in CFS/ME
Dr Andreas Finkelmeyer, University of Newcastle

Key point summary:

- A significant increase in brain grey matter (GM) volume and significantly lower WM volume, but similar cerebrospinal fluid volume was found in patients with CFS/ME compared with healthy controls.
- The specific brain regions affected are responsible for interoception (the relationship between bodily sensations and emotional experience), salience (focusing attention) and valence processing (feeling pleasant or unpleasant, and feeling activated and energized).
- These findings need to be replicated with larger sample sizes.

The presentation began with a quote from Natelson (2013):

“These studies led to our current working hypothesis - that a subgroup of patients with CFS/ME has an underlying neurological disease which leads to the symptoms of fatigue and cognitive dysfunction.”

Dr Finkelmeyer then displayed a list of brain morphology studies of patients with CFS/ME compared with healthy controls to demonstrate that there have been very few studies so far with findings that have been replicated and that there are contradictory results:

- Okada (2004): reduced GM in the prefrontal cortex, related to performance status; no white matter difference
- De Lange (2005): Global GM volume reduction, related to physical activity
- De Lange (2008): Increase in GM volume following CBT (prefrontal cortex, global)
- Barnden (2011, 2015): no global or regional volume differences between groups; various interactions
- Puri (2012): reduced GM & WM in occipital regions
- Zeineh (2015): global WM reduction; localized cortical thickness (GM) reductions
- Van der Schaaf (2016): no global or regional GM differences, but association with chronic pain in prefrontal cortex

The aim of this study was to investigate differences in grey matter (GM) and white matter (WM) volume between CFS/ME patients and healthy controls using modern voxel-based morphometry (VBM) methods.

In VBM, MRI scans are analysed by software to more clearly define the areas of white matter and grey matter and to morph them to a common template. Subsequent smoothing of the images makes the scans from individuals easier to compare mathematically, and hence aids the statistical analysis of GM and WM volumes.

In this study, 42 patients (32 female), with a mean age of 45.2 years who fulfilled the Fukuda criteria, with co-morbid psychiatric conditions excluded, were compared with 28 healthy controls (19 female), with a mean age 48.4 years.

All of the MRI scans were produced by the same 3T Philips Achieva MRI scanner at Newcastle Magnetic Resonance Centre, using a standard T1-weighted anatomical scan sequence. Pre-processing used the Computational Anatomy Toolbox in SPM12. Voxel-wise statistical comparisons controlled for total intracranial volume, age, and sex.

Whole-brain differences

When whole-brain volumes were compared (see figure 1), there was a significant difference in the total intracranial and WM volumes, with patients having lower volumes than healthy controls. This could just have shown that the study had recruited patients with smaller head size (total intracranial volume) than controls, but when the results were corrected for this variable by dividing the segment volumes by head size, a significant increase in GM and significantly lower WM were seen, but similar cerebrospinal fluid (CSF) volume (see figure 2).

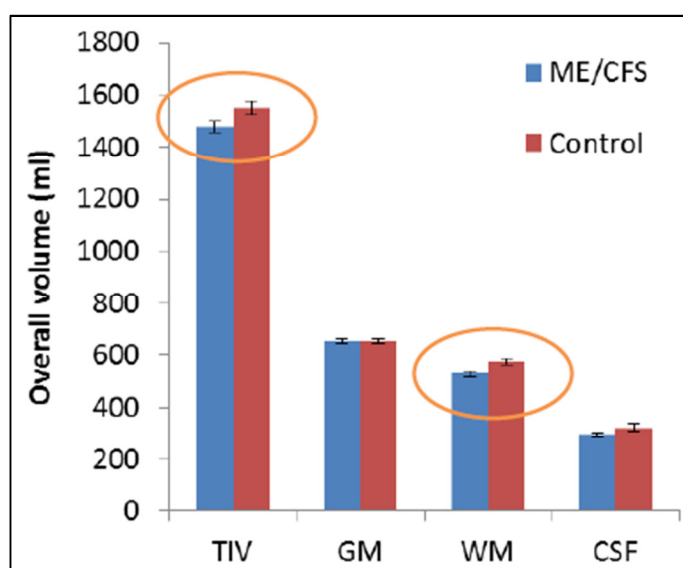


Figure 1: Comparison of whole-brain volumes in CFS/ME patients and healthy controls

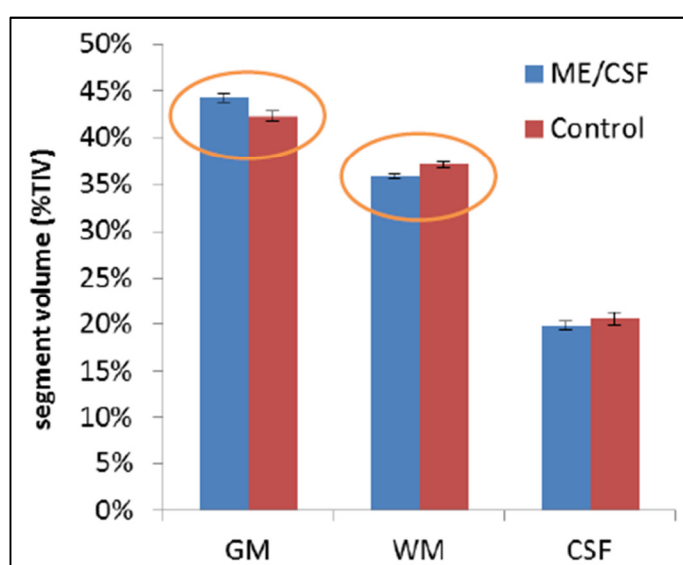


Figure 2: Comparison of segment volumes (%TIV) in CFS/ME patients and healthy controls

Regional brain differences

Regionally there were increases in GM in CFS/ME patients, with clusters in the right posterior insula, the right amygdala, left amygdala, and the right medial temporal lobe.

These findings have not been reported by previous studies and so have not been replicated, so they should be interpreted cautiously, but the potential clinical significance is that the insula is concerned with interoception, (the relationship between bodily sensations and emotional experience), and the amygdala with salience (focusing attention) and valence processing (feeling pleasant or unpleasant, and feeling activated and energised).

There were decreases in WM in the patients, primarily in the corticospinal tracts of the midbrain, the brainstem (specifically in the pons), and the median temporal lobe. These brain structures are involved with pain perception, which may be the clinical significance of these differences.

A previous study in CFS/ME patients by Barnden et al in 2011 found midbrain WM volume decreased with CFS/ME duration, and pulse pressure related to signal intensity in the brainstem. The reduced volume in anterior temporal WM (uncinate fasciculus), perhaps confirms speculations regarding reductions of crossing fibres (Zeineh et al, 2015) presented by Prof Montoya at the 2015 CMRC conference.

Conclusion

Dr Finkelmeyer concluded by pointing out that these interesting findings need to be replicated with larger sample sizes, and although VBM has been around for quite some time, it is still being developed in terms of the software used, and the methodology needs to be consistent.

A limitation of this research method is that segmentation based on image contrast may produce results that are not actually down to brain matter volume. It is not known whether differences seen between CFS/ME patients and healthy controls are a result of the illness itself and whether and how they relate to the clinical symptoms.

Immune-pain interaction following exercise in CFS/ME: associations between exercise-induced hyperalgesia, complement system and elastase activation

Dr Andrea Polli, University of Brussels

Key point summary:

- The complement system and elastase have been found to be associated with fatigue and/or pain.
- Participants of this study carried out an exercise test which was repeated a week later.
- Blood samples and pain pressure threshold (PPT) before and after each exercise test and showed that PPT and complement system changes were only associated in patients with CFS/ME and not healthy controls.
- In healthy controls PPT increased, whereas this decreased in CFS/ME patients.
- There were no changes in elastase levels, or differences between patients and controls.

Pain is the second most important symptom to people with CFS/ME and it can often be more distressing than fatigue. There is possibly a shared underlying pathology between fatigue and pain, as 70% of patients also have fibromyalgia, which is characterised by widespread, disabling pain.

Although interleukins were thought to be a good measure of immune system activation, systematic reviews have shown that they do not explain CFS/ME symptoms and exercise-induced changes, whereas the complement system and elastase have been found to be associated with fatigue and/or pain. Studies have shown that C4a, but not C3a or C5a, increased six hours after sub-maximal exercise.

Method

This study recruited 22 patients with CDC-defined CFS/ME and 22 healthy controls. In experiment 1, the participants performed a standardised test known as sub-maximal exercise or aerobic power index using an exercise bike, cycling with a 60-70 pedalling rate per minute, and the resistance of the machine was increased by 25 Watts minute by minute until the participant reaches 75% of their target heart rate.

Before and after the exercise test, pain pressure threshold (PPT) was measured. PPTs are thought to be an effective way of measuring central sensitisation and central nervous system hyperexcitability. Blood samples were also taken before and after, to measure complement system and elastase activation.

In experiment 2 a week later, the method and measurements in experiment 1 were repeated, but in order not to worsen the participants' CFS/ME symptoms more than necessary for the experiment, the exercise was self-paced and physiologically limited. This means they were able to estimate themselves how long they could cycle for, and their heart rate and anaerobic threshold were monitored so that the exercise could be stopped in order to prevent symptoms worsening.

PPT responses were similar for both experiments but were significantly different between healthy controls and CFS/ME patients (see Fig 1).

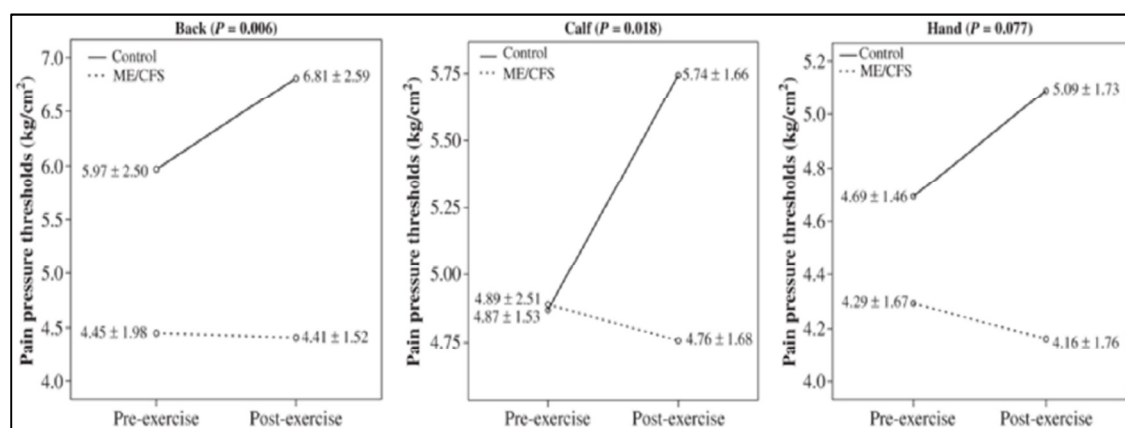


Fig 8: Pre- and post-exercise PPT in CFS/ME patients and healthy controls in experiment 1

For the healthy controls, PPT increased after exercise, meaning that more pressure was needed in order to reach their threshold, whereas for CFS/ME patients, the threshold decreased. The changes made to the exercise test in experiment 2 were unsuccessful in preventing worsening of CFS/ME symptoms.

Immune system changes did not differ between patients and controls but the complement system activation significantly decreased after exercise in both experiments for both groups. There were no changes to elastase levels after both types of exercise.

When correlation analysis was performed, PPT and complement system changes were only associated in patients with CFS/ME and not healthy controls (see Fig 2). Regression analysis found that changes in the complement system can partly explain (24%) the change in PPT results.

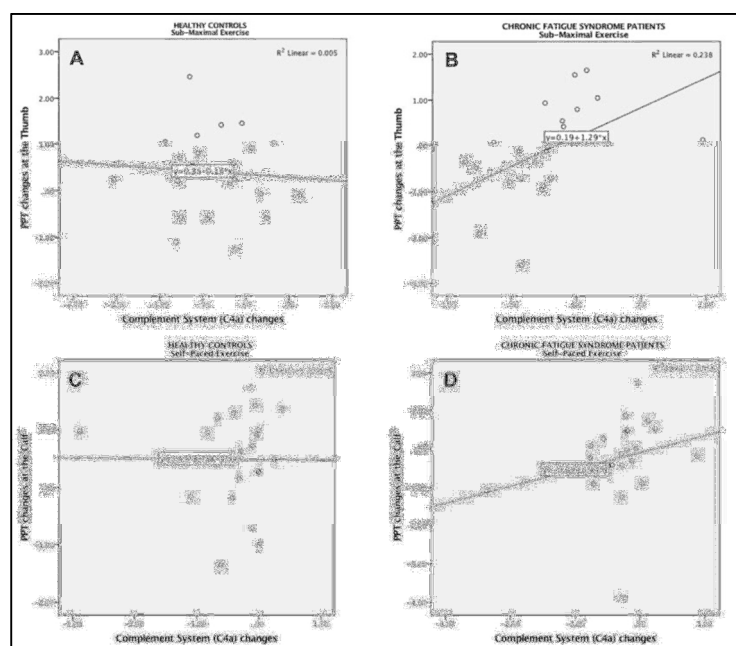


Fig 9: Correlations between exercise-induced changes in PPTs and changes in the complement system (C4a)

Limitations of the study were that it was only a small sample and very indirect measures were used and the results were not straightforward. Interesting findings include a moderate or strong association, despite using these indirect measures. Changes in the complement system seem to explain part of the change in PPTs after the sub-maximal exercise test in patients with CFS/ME.

A self-paced, physiologically limited exercise test had similar effects in CFS/ME patients to a more demanding sub-maximal exercise test at 75% of the target heart rate suggesting that the type of exercise is perhaps not important but that any form of exercise can cause immune system changes in people with CFS/ME.

CHILDREN AND YOUNG PEOPLE

Improving quality of care in paediatric CFS/ME

Dr Sarah Knight, Clinical Neuropsychologist, Murdoch Children's Research Institute

Key point summary:

- There are limited validated outcome measures for paediatric CFS/ME.
- There are limited evidence-based treatment options for paediatric CFS/ME.
- Time spent waiting for a diagnosis and access to specialist care can be protracted.
- Variability in clinical approaches to diagnosis, treatment and management is considerable.
- More research using age-standardised objective and subjective measures is necessary.

Dr Knight coordinates a clinical research program for paediatric CFS/ME in Australia. In collaboration with neuroimaging, neuropsychological, sleep, educational and medical specialists across the country, this growing multisite program has a particular emphasis on actively facilitating the translation of research into clinical practice.

She explained that her presentation would highlight the challenges of implementing an outcome measurement system for paediatric CFS/ME, and the scope of the problem in this area in Australia.

According to a study by Bakken (*BMC Medicine*, 2014), adolescence (10-19 years) represents one of two age peaks in the incidence of CFS/ME (the other being a 30 to 39 years) – see Figure 1. However, a range of studies gives a wide variance of incidence, from 0.0006 to 2%.

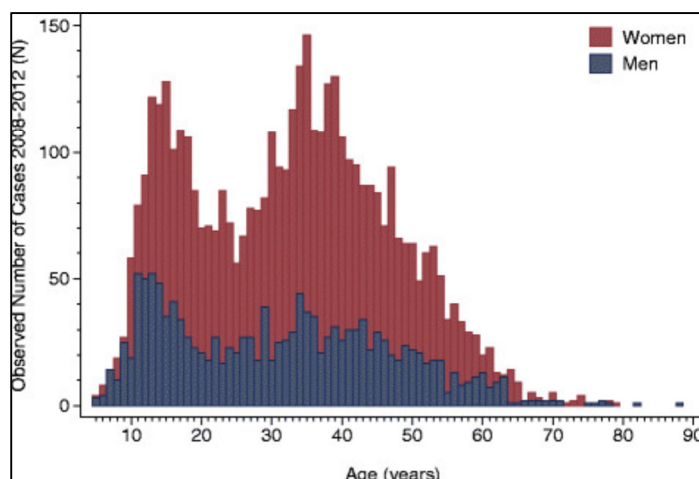


Figure 1: Bakken et al. 2014

Development of CFS/ME in adolescence presents on a background of rapid physical, psychological, and social developmental changes. This is already a vulnerable period, with rapid maturational changes in the areas of wellbeing and mental health, education and

schooling, cognitive capacities, sleep, family and peer relations, independence, self and identity and sexuality, and the future long-term risk to educational attainment and/or employment is self-evident. Compounding this, the variance in symptom presentation presents numerous challenges to measurement and management.

Dr Knight gave an overview of the functional impact of paediatric CFS/ME. The average amount of time away from school for students diagnosed with CFS/ME has been estimated to be one year across their school life (Rangal et al. 2000) and almost half of students with CFS/ME attended school for only 20% (or less) of the expected attendance (Crawley & Sterne, 2009).

She also highlighted that 41% of adolescents with CFS/ME attended less than 40% of school (Bould et al. 2013) and that the impact of physical and psychological function is considerable:

- most children with CFS/ME have poor physical function with up to 57% bed bound at some stage (Crawley and Sterne, 2009)
- girls with CFS/ME are three to four times more likely to experience anxiety (Crawley et al, 2009) and rates of depression are 10 times higher in adolescents with CFS/ME (Bould et al. 2013).

She then gave an overview of the Murdoch Children's Research Institute's aims and approach – see Figure 2.



Figure 2: Aims and approach of Murdoch Children's Research Institute

There are a number of challenges to achieving this. Citing numerous papers, Dr Knight highlighted that “the poor quality and acceptability of available measures (PROMs) limits current recommendations for the patient-reported assessment of paediatric CFS/ME of relevance to routine practice, service evaluation and research settings” (Crawley et al,

2009) and that “evidence for instrument properties in children with CFS/ME was lacking, despite the high proportion of studies with this population.” (Crichton et al, 2015). She posed a number of questions with regards to the scope of the problem in paediatric CFS/ME in Australia:

- Are there any paediatric-specific, evidence-based treatment options?
- Are clinical approaches to diagnosis and management consistent in Australia?
- What is the incidence of paediatric CFS/ME in Australia?

Having undertaken a systematic review (*Journal of Adolescent Health*, 2013) – and highlighting the variable methodological quality of the studies reviewed – Dr Knight found limited evidence-based treatment options, and delays in diagnosis and treatment amounting to more than 12 months. There was also considerable variability in diagnostic and treatment approaches – see Figure 3.

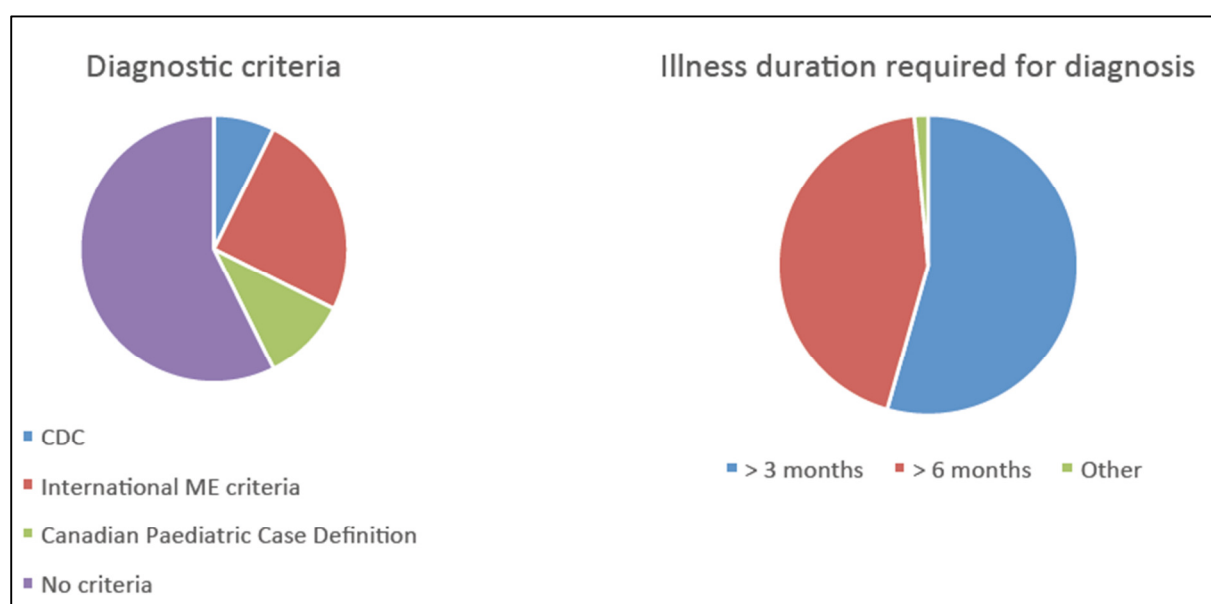


Figure 3: Wide variability in diagnostic criteria

Brain and cognitive function

Difficulties with memory, concentration and information processing are well evidenced in adults with CFS/ME, along with decreased grey and white matter volumes and metabolic differences.

Less is known about these issues in adolescents with CFS/ME, though there is some evidence of metabolic differences and reduced functional connectivity related to fatigue and pain symptoms. More than 80% also report cognitive problems and there are significant IQ differences between adolescents with CFS/ME and healthy controls.

Dr Knight gave an overview of study funded by ME Research UK in which she and her team are investigating brain and cognitive functioning in adolescents with CFS/ME using brain imaging technology. The study design is shown in Figure 4.

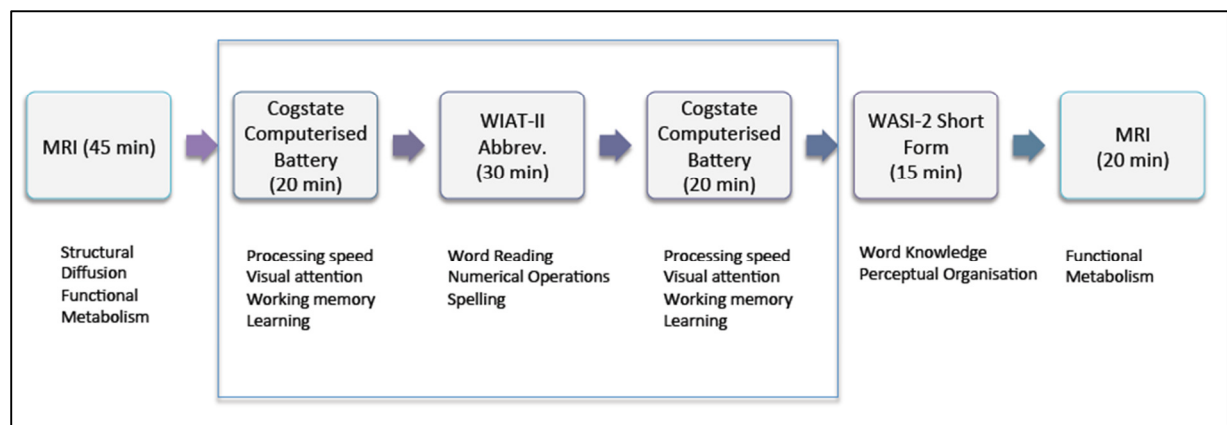


Figure 4: Study design

This work is being undertaken as part of the Institute’s long-term goal to better understand paediatric CFS/ME, and ultimately leading to improvements in quality of care.

This goal relies on clinical translational research programs; international consensus in terminology, diagnostic criteria and the definition of recovery for use in paediatrics; standardised paediatric minimum data set; use and development of age-standardised, validated subjective and objective outcome measures; exploration of the complex interactions between systems (eg. brain) and symptomatology (eg. cognition) and longitudinal studies to map illness trajectory.

Investigating the treatment of Paediatric CFS/ME
Prof Esther Crawley, University of Bristol

Key point summary:

- CFS/ME in children is important to study, as it is a disabling illness severely affecting school attendance and teenage development, as well as being costly to the country in terms of health resource usage.
- There are problems with investigating treatments because there is no clear and consistent way of defining and measuring recovery.
- The treatment outcomes wanted by children and their parents differ from those of clinicians, so a patient-reported outcome measure has been defined with input from children and their parents.
- The MAGENTA and FITNET trials are explained, with a progress report.

Prof Crawley talked about a variety of methods her clinic has used in working with children and their parents to try to improve treatment outcomes.

In children, CFS/ME is very common. It affects 1% of secondary school children, causing them to miss 20% of school, and 0.1% of 13 year olds are housebound. The illness is also very disabling, as within specialist services, mean school attendance is only 40%. 30% of the children also develop anxiety or depression in addition to their CFS/ME.

The illness has a high financial and emotional impact on families, and high healthcare costs. In the years prior to CFS/ME diagnosis, children seeing their GP more often, receive more prescriptions, and have more investigations, as shown in the poster by Simon Collin.

The problem

To design trials in children there needs to be better Patient Reported Outcome Measures (PROMs) and Minimally Clinically Important Difference must be defined. All children and parents ask questions about recovery such as “Is my child going to get better, and when?” and we cannot answer this question because there is no definition of recovery and what factors affect it, said Prof Crawley.

Systematic review: recovery definitions

A review has been carried out looking at every trial, prospective study, and epidemiological study which had a measure of recovery in paediatric CFS/ME. Some trials use a single measure such as school attendance or fatigue, whereas some use multiple measures. The more robust studies use a composite measure to define recovery.

What children and commissioners need to know

Children who have access to specialist treatment have over 60% chance of complete recovery at six months, using a composite score for recovery, whereas those without have a less than 20% chance. This means we have an obligation to provide specialist treatment, says Prof Crawley. Children are much more likely to recover than adults, though it is not known why this is.

How did the children/parents define recovery?

This was difficult to ascertain because children were reluctant to talk about recovery because it made them depressed about what they could no longer do, while those that had recovered did not want to be reminded of how ill they had been. Each child has their own definition of recovery, such as being able to play sports again or going back to school. There are different ways of defining recovery but it would be ideal to have either a combined score or a defined single question on recovery.

Developing a new PROM

When developing an outcome measure, we need to understand how patients conceptualise the illness, then define the domains and the questions, as shown in figure 1.

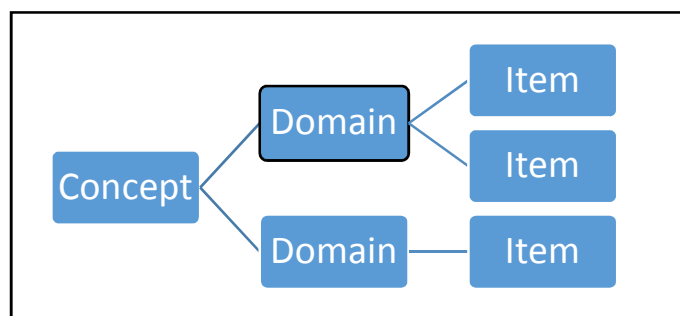


Figure 10: Process for developing a new PROM

Methodology

A variety of approaches were used but mainly semi-structured interviews which were child-friendly and interactive. The ideas were then discussed with a Young Person's Advisory Group (YPAG).

It was found that children with CFS/ME and clinicians have a completely different conceptual model of CFS/ME. The children's model is linear: they see it as getting CFS/ME and feel terrible, which then causes a whole raft of symptoms, causing them to reduce physical activity and social participation, which in turn has an effect on their wellbeing. Other factors played a part, such as how people engage with them at school. Clinicians have a different model and think it is more complex, with each factor affecting others.

When children were interviewed about factors which were important to them, they come up with a huge number of domains they wanted measuring, and this is difficult to put into a questionnaire as it would be too lengthy. Interestingly none of them mentioned biological

measures. A method called card-ranking was used to establish which the most important factors to children were.

Recruitment of participants was through the paediatric CFS/ME services, so they fulfilled the NICE criteria for mild-moderately affected. Twenty one children (16 females and 6 males) with a mean age of 14.4 years participated. Quantitative analysis was carried out on the card-ranking exercise by the children and their parents of what they wanted to see being measured as treatment outcomes.

Results

An example was shown of the card-ranking exercise by a 15 year old girl with CFS/ME and her mother to compare their different priorities. The top priorities of the girl were the measurement of symptoms, but also the fluctuating nature and instability of the illness. The mothers of the children were more concerned with mood and self-esteem. Overall, there were similarities in how the children and parents prioritised measuring symptoms, tiredness, and payback (or crashing), but there were also differences.

There were gender differences in priorities for measuring outcomes, with “symptoms” being more important to girls and “activities and hobbies” being more important to boys. There were also age differences, with “mood” being ranked higher by older children (14-15 years old) and “family impact” being more important to younger children (12-13 years old).

A great deal was learnt about the effects of illness through the interviews. When using the card-ranking to prompt more questions asked about symptoms, one participant explained, “They’re always there and on a bad day they get worse”. When asked about an outcome measure for activities and hobbies, another participant said, “I think being able to do a certain activity, if I’m doing it for longer, or having more energy to be able to do it”. This shows how complicated the problems of measurement are. Are we going to measure how many activities a child can do, how long they can do it, or how frequently they can do it?

On why symptoms are important to measure, one participant explained, “so I can try and get rid of the symptoms so that I can get to school more and see my friends”. The participant’s mother said “I think the confidence is affected by her symptoms, so she’s not sure she can anymore”.

The final conception model with the domains and the questions asked for each was then shown. The four domains identified are: Hobbies and leisure, Friends and family, Fatigue, and Symptoms. This new PROM is now finished and is entering the final testing phase. It has been designed to be completed online.

In research, results are usually presented in terms of whether there is a significant difference in outcomes but the MCID concept instead looks at whether what we are measuring is as an important difference to patients. There are three methods for this: the consensus method, the anchor method, or interviews, and this study used all three of these.

The consensus method involves a group of clinicians making a decision on behalf of the patients on what change is actually important. The anchor method looks at improvement by

comparing the outcome scores before and after treatment to observe a change, and the SF-36 scoring was the most popular with patients.

The MAGENTA trial

The aim of the trial is to investigate the effectiveness and cost-effectiveness of Graded Exercise Therapy (GET) as there are no RCTs of GET in children currently, even though NICE recommends it. In Prof Crawley's experience, children choose GET when it is available. It is difficult to recommend it, without being certain that it is effective.

The feasibility phase has just been completed. The trial used integrated qualitative methods, which means that throughout the trial the children, parents and clinicians were consulted about the interventions and trial process.

The aim of both interventions in the trial is to convert the boom-and-bust pattern to a stable baseline of activity, which involves cutting back on activity, which is not popular, as the children want to keep doing their physical hobbies, such as sports and ballet. All children are offered advice and symptom control, for example sleep and medication, and are then part of treatment arms:

- **Activity management:** All activity – mostly cognitive activities (school, school work, reading, socialising, and screen time).
- **Graded Exercise Therapy:** detailed assessment of current physical activity and focusses on evening out physical activity and then very slowly increasing.

It is now one year into the trial, and recruitment has been excellent, and the children like both treatment arms and have been sticking to the treatments well. Retention and follow up have also been good. Children also report that they have liked being part of the trial as they want to help improve treatments for other children with CFS/ME in the future. A data safety monitoring committee report recommended MAGENTA moved to full study

The FITNET-NHS trial

This is a new HTA funded trial and recruitment begins in November 2016. Many children do not have access to a specialist service; families are travelling four or five hours each way and staying in a hotel to attend the service in Bristol, which is expensive and also very tiring for the children with CFS/ME, and because of this Prof Crawley wanted to develop an intervention which can be delivered locally to the children.

FITNET is an abbreviation of Fatigue in Teenagers on the interNET, and this was originally published in the Lancet (Nijhof, 2012). It showed that 63% of the children in the FITNET arm had recovered at 6 months compared with 8% in the "standard medical care" arm. This might be a way of providing an intervention for adolescents with CFS/ME in the UK.

Children are referred to the study via their GP, then assessed by a paediatrician for their eligibility, and if they wish participate in the trial are randomly assigned to one of the two treatment arms: Activity management and behavioural therapy via Skype; or FITNET-NHS internet CBT modules. The trial will be the largest in CFS/ME in adults or children, at 660 participants.

Involving severe and very severely affected CFS/ME individuals in research: a clinician's view point
Victoria Strassheim, Newcastle University

Key point summary:

- Involving severely affected patients in research is difficult because they are too unwell to take part.
- Results may only really reflect the better-functioning patients with severe CFS/ME as they were more able to complete the questionnaires and home visits.
- Semi-structured interviews were preferred over lengthy questionnaires
- Reaching these patients to include them in studies is hampered by the standard ethics rules that patients must volunteer themselves rather than being directly approached.
- These problems need to be overcome in order to study severe CFS/ME and ensure that research is including the full spectrum of CFS/ME severity

Victoria Strassheim is a chartered physiotherapist who has a background in neuro rehab, and has been working on a project to define the prevalence of severe CFS/ME in the northern region of the UK, including understanding the clinical characteristics of severe CFS/ME and the relationship with cognitive and autonomic dysfunction. The project used Prof Diane Cox's descriptors for severe CFS/ME in the 2002 CMO report, so patients who are housebound, wheelchair-bound and bed-bound. Victoria shares her experience of involving people severely affected as research participants for this project in this talk.

Population breakdown

The size of the population in the geographical area of the study in 2011 census is just over 2.5million. If the estimated prevalence of CFS/ME as 0.4% is correct, there are roughly 10,000 people affected by the illness in the region. As 25% of people with CFS/ME are thought to be severely affected, this gives a figure of around 2,500 with severe CFS/ME.

Recruitment process

In research, potential participants cannot be approached; they have to apply to be participants themselves. The research group advertised for participants through specialist services, GP practices, local and national charities, and social media. A questionnaire pack was sent out to those interested, including an "expression of interest", a patient information leaflet, and three questionnaires. These questionnaires were demographics, the De Paul questionnaire, and a Barthel questionnaire to measure performance in activities of daily living.

Initially, 58 questionnaire packs were sent out and 38 were received back. It was taking a long time to recruit enough participants, so local support group ME North East was approached to see if they had any advice on where recruitment was going wrong. It was

suggested that there was too much involved for people with severe CFS/ME in sending back and forth information. The research team asked the ethics committee if they would approve of a substantial amendment to the process by sending questionnaire packs to ME North East for them to then send on to patients in their membership. 425 packs were sent out by ME North East but only 25 were returned, taking the number of participants to 63.

This obstacle of patients not being well enough to participate needs to be overcome somehow in order to make sure CFS/ME research includes severely affected patients. This may need to mean waiving some of the consent issues so that patients can be contacted directly.

Barthel Functional Outcome Questionnaire

A score of 12 is the pivotal score at which point a person moves from independence to dependence on someone else for their personal care and mobility. Of the 48 Barthel questionnaires returned, 30% scored 12 or less. Scoring less than 17 but more than 12 suggests help is needed to perform activities of daily living, and this applied to 67% of the responders.

De Paul Symptom Questionnaire

This is a comprehensive questionnaire which can ascertain which criteria fits the patient, out of the CDC, Canadian and Fukuda criteria. It involves scoring 54 symptoms on a scale. A reason for not returning the questionnaire may be that it is too long for someone who is severely affected, so the questionnaires returned will only reflect the less severely affected of the patients with severe CFS/ME.

From the 56 De Paul questionnaires returned:

- 60% fulfilled both the Fukuda and Canadian diagnostic criteria
- 18% fulfilled either the Fukuda and Canadian diagnostic criteria, but not both
- 22% did not fulfil either of the diagnostic criteria

This suggests that 40% of the patient group require further investigation to produce a definitive diagnosis. There were a total of 65 different conditions in the participants' past medical history, and there were 16 different current comorbidities, so it is possible that these people have other conditions that have gone undiagnosed.

Phase 2: home visits

The next stage of the study involved visiting five patients in their home four times. 3 patients (60%) managed all four visits and the other 2 patients (40%) only completed three visits, with one not managing to complete the full session and the other having to decline on the day of the visit. The patients wanted to participate as much as they could and they tried really hard to but their illness limited their ability to participate.

The first of the visits was for the consent procedure and the autonomic testing. Visit 2 was neurocognitive testing. Visit 3 was semi-structured interviews, and the final visit was a physical physio assessment to find out what the patients were physically able to do.

The patients found it very upsetting to realise through the assessments how bad their symptoms are affecting them, and would have been left having to cope with the emotions themselves after the visit, so a clinical decision was made not to do some of the assessments for some participants.

The semi-structured interviews were popular as it gave the patients the opportunity to tell the world their story and what their life is like, when they are usually not heard. The participants that lived with their parents were more able to complete the assessments, possibly because they were receiving much more support.

Conclusion

Confirmation of the diagnosis of the third of the participants who did not fulfil the diagnostic criteria to verify their CFS/ME diagnosis is needed. More investment into accessing patients with severe CFS/ME is needed, with allocation of advocates to aid involvement.

The visualisation of CFS/ME's invisibilities

Dr Juliet Chenery-Robson, University of Sunderland

This project, funded by the Arts and Humanities Research Council, aimed to develop a range of strategies for visualising the invisible illness CFS/ME through photography, text and participatory practice.

The chronically ill CFS/ME sufferer experiences invisibility on four interlinked fronts, said Dr Chenery-Robson: physical, social, medical, and political invisibility. The main aim of the research was therefore to create work, in participation with 16 people with CFS/ME, which could be used as a tool to raise awareness of CFS/ME's invisibilities and communicate an understanding of CFS/ME to public and medical audiences.

By exploring the many layers that constitute CFS/ME (history, symptomology, names, treatment) and its personal impact upon sufferers – through a combination of photography, text, audio, SenseCam, family album photographs and Google Earth images – the research tests different methods of using metaphor to visually represent CFS/ME's invisibilities. To reflect CFS/ME's many facets a pluralistic methodology was adopted, which was both directed and participatory, combining elements of reflective practice, interpretive phenomenology, and narrative medicine.

Following a broad range of UK and international exhibitions and presentations, written evaluations have been collated from the public, medical practitioners, academics, curators, CFS/ME sufferers and their families/carers. These evaluations have proven that a multiplicity of creative practice can provide a more nuanced and flexible method of visualising aspects of CFS/ME's invisibility. It has also helped provide CFS/ME sufferers with a 'voice' by which to communicate the many problems they face as they try to cope with a life that is disabled by CFS/ME.

An exploration of the multi-faceted strategies developed for visualising aspects pertaining to CFS/ME's invisibility demonstrate how photography, used in conjunction with text, can contribute to a wider public/medical understanding of CFS/ME.

Investigating the possibility of a role for mtDNA variation in CFS/ME

Joanna Elson, Mitochondrial Research Group, Institute of Genetic Medicine, Newcastle University

Mitochondria are subcellular organelles that are responsible for energy production. They contain a small chromosome of their own, commonly referred to as mitochondrial DNA (mtDNA). Mutations of the mitochondrial chromosome are a well-documented cause of inherited disease, mtDNA mutations affect around one in 5,000 individuals in the UK, with around 250 disease-causing mutations having been identified.

The first aim of this project was to see if known mtDNA mutations could be detected in people with CFS/ME. To date, more than 300 complete mtDNA sequences from CFS/ME patients have been fully sequenced without finding any confirmed mtDNA mutations. The second aim of the study was to see if population variants (polymorphism) play a role in the susceptibility to CFS/ME.

A large number of studies have suggested that mtDNA population variation is important in susceptibility or course of common complex diseases, such as diabetes and multiple sclerosis. Most of these studies have used the traditional haplogroup association method. However, inconsistent results are commonly seen when comparing studies using this form of analysis. In this study we use a novel “mutational load hypothesis” to investigate a possible role for mtDNA population variants in susceptibility to CFS/ME.

The study has used two well-characterised clinical cohorts of CFS/ME patients, one from the UK and the other from South Africa. The mutational load hypothesis considers the collective effect of all non-synonymous variants on an individual’s mtDNA. As such, this method incorporates rare population variants which have a higher likelihood of being deleterious.

Additionally, it has greater statistical power to detect differences between affected and unaffected cohorts, as it describes the mtDNA variation of an individual in a single metric, hence allowing cohorts to be compared using parametric statistics in a single test.

PLENARY SESSION

Fatigue in Primary Sjögrens Syndrome is associated with lower levels of pro-inflammatory cytokines

Dr Nadia Howard-Tripp, NIHR Academic Clinical Fellow in Rheumatology

Primary Sjögrens Syndrome is a chronic autoimmune inflammatory condition with 70% of patients suffering from fatigue alongside other systemic manifestations.

“The pathophysiology of fatigue is unclear, no biomarkers have yet been identified and it is challenging to study,” explained Dr Howard-Tripp. “We think there may be an immunological or inflammatory basis to this.”

This is partly because the phenomenon of post infectious fatigue and the fact that it’s incredibly common in a number of autoimmune diseases. And there is “a huge body of research has suggested that there is some immune dysregulation of some sort in patients who have CFS/ME.”

Using Sjögrens as a model of fatigue they recruited 159 patients from the UK Primary Sjögrens Syndrome registry along with 28 healthy controls. Fatigue questionnaires were completed, clinical and demographic data collected and levels of 24 cytokines were measured. The research team then looked at the differences between Sjögren patients and controls, then the association between cytokine levels and fatigue levels and, using logistic regression analysis, they teased out which variables were associated with fatigue.

“We classified the Sjögrens patients into minimal, mild, moderate and severe fatigue based on the PROFAD physical fatigue score which is validated for use in Sjögrens,” said Dr Howard-Tripp. Patients had similar demographics, disease duration and importantly similar medication use.

Of the 24 cytokines, 4 differed between patients and controls: IP10 (interferon gamma producing protein), interferon gamma and lymphotoxin alpha and TNFa.

Explaining the results, Dr Howard-Tripp said: “Its potentially a bit counter intuitive, in that as fatigue level increase the level of these pro inflammatory cytokines is actually decreasing. It was a slight decrease, she said, but statistically significant.

The team performed a logistic regression analysis using the array of data they had collected including levels of all 24 cytokines, white cell counts, measures of clinical disease activity as well as patient reported anxiety, depression and pain.

“We then looked to see if the computer could predict the level of fatigue,” she explained. “Using all of that information, in 67% of cases it could predict the level correctly.”

Continuing to refine their findings, they stripped down the data to reveal a reduced model in which IP10, interferon gamma, pain and depression were able to predict fatigue with the same accuracy.

“It is worth mentioning that anxiety and depression alone and just the cytokines alone were not as good. It was the combination of both that worked best,” she said.

Looking again at their results where a decrease in cytokines is associated with increased fatigue, Dr Howard-Tripp said, “What we wondered was that in the context of a constant immune challenge such as in Sjögrens, where there is always a chronic low level of inflammation, could that cause an increase of the regulatory or the negative feedback mechanisms.” This in turn could cause inappropriate negative feedback and a reduction in the pro inflammatory response.

“And that could be either a by-product or a cause, that blocks the recovery that some patients have and leads to the persistent fatigue,” she said. The team have now joined forces with biochemists and scientists with a special interest in cytokines to work out why this might be happening.

Though this is a speculative model, explained Dr Howard-Tripp, there is supporting evidence for their theories. She cited the work of Hornig et al (2015) which looked at short and long duration (less than 3 years and more than 3 years) in CFS/ME and controls. With a far larger cohort, these researchers found that a number of pro inflammatory cytokines were lower in the CFS/ME patients who had were ill for longer. “Perhaps there is a temporal process going on that means initially there are higher levels of inflammation but over time the inflammation is decreasing,” she said.

Referring to a slightly older Dutch study which looked at adolescents compared to controls, researchers found IL6 and TNFa were lower in those who had CFS/ME while IL10, which is typically anti-inflammatory, was higher.

Dr Howard-Tripp highlighted the limitations of their own study, saying that cytokines fluctuate widely at a given point in time, the cohort included females only and the model is speculative rather than proven. Further work is required, she said, to examine role of anti-inflammatory and regulatory pathways to see if they do have a role in fatigue.

The role of autonomic function in exercise-induced endogenous analgesia: a case-control study in CFS/ME and healthy people

Prof Dr Jessica Van Oosterwijck, Pain in Motion international research group, Ghent University, University of Antwerp

Key point summary:

- In healthy people, the autonomic nervous system is rebalanced by increasing the activity of the parasympathetic branch, making the heart rate decelerate until it returns to pre-exercise levels.
- Exercise activates endogenous analgesia in healthy individuals which results in higher pain thresholds and experiencing less pain, a phenomenon known as exercise-induced analgesia.
- In CFS/ME, heart rate recovery following exercise is delayed, the parasympathetic modulation or reactivation during recovery from exercise is delayed and reduced in magnitude, and exercise-induced analgesia is impaired.
- Drug treatments in combination with graded exercise therapy may improve baroreceptor reflex sensitivity and parasympathetic tone during exercise recovery in CFS/ME patients, which in turn may improve exercise-induced analgesia.

The autonomic nervous system is the main regulatory system of the body and influences vital functions such as blood pressure, heart rate, respiration and temperature. It consists of two branches, the sympathetic and parasympathetic branch. Both branches are continuously active; however, one branch can be dominant active over the other.

The activity of the parasympathetic branch dominates when we are relaxed, as in rest-and-digest, whereas the sympathetic branch is dominant when we exercise or perceive danger, as in the fight-or-flight response. When the sympathetic branch is activated, the heart rate accelerates and more blood is directed to the skeletal muscles so they can use the energy from the oxygen within our blood to fight or flight.

Although today we do not often have to fight or run away in order to survive as was the case in prehistoric times, we still come across stressful situations. So we still go running or perform other physical exercises on a regularly basis because we know this has beneficial effects for our health.

When we undergo stress or exercise, the sympathetic branch is activated and its activity will dominate that of the parasympathetic branch. In order to recover from exercise and restore homeostasis, the activity of the two branches of the autonomic nervous system are rebalanced following exercise by reactivating the parasympathetic branch. This will lead to deceleration of the heart rate returning it to pre-exercise levels, which is known as heart rate recovery.

A previous study by Prof Dr Van Oosterwijck (unpublished) and her team showed that heart rate recovery is delayed in CFS/ME and that the parasympathetic reactivation during recovery from exercise is delayed and reduced in magnitude in those with CFS/ME. We also

know from other studies that healthy people feel less pain when they exercise, but that this phenomenon – termed exercise-induced analgesia (EIA) – is impaired in CFS/ME patients (Van Oosterwijck et al., 2010). Their pain stays the same or even worsens following exercise. Because the mechanisms of this phenomenon are not fully clear, the current study investigates the role of autonomic (dis)function in EIA.

Methods

Twenty CFS/ME patients diagnosed with the CDC 1994 criteria and 20 sedentary healthy controls took part in this study. All participants attended a familiarisation session where demographic or disease related data was gathered; patients were checked whether they also met the more recent 2011 Canadian Criteria, and were found to do so. This session was also important to reduce stress on the day of the experiment due to the unfamiliar environment, which could have confounded the findings, as stress triggers the activation sympathetic nervous system. Participants were guided through the lab, the full test procedure was explained, and the different assessment methods and materials were shown and tried out.

During the second visit, participants performed a submaximal bicycle exercise test with continuous cardiorespiratory monitoring to induce EIA. The Aerobic Power Index test was performed as described in our previous study (Van Oosterwijck et al, 2010) and in the presentation by Dr Polli.

In summary, the exercise protocol commenced at 25W and the workload (W) was linearly increased by 25W/minute maintaining a cycling rate of 70 rotations/minute until 75% of the age-predicted target HR was reached. The exercise test was concluded by a short cooling-down of 30 seconds, during which the subject kept cycling against a resistance of 25W, to prevent venous pooling. Immediately following the exercise, subjects were asked to assess their perceived exertion using the Ratings of Perceived Exertion (RPE) Borg scale.

Prior to exercise testing (at rest) and during the subsequent recovery period physiological measures of autonomic function were performed to assess respiration rate (RR), blood pressure (BP) and heart rate variability (HRV). HRV describes the beat to beat variations (i.e. time domain parameter) in heart rate measured by electrocardiogram (ECG).

The HRV gives an indication of the ability of the nervous system to respond and recover from physical or physiological stressors. The HRV can also be transformed into frequency parameters, in which the parasympathetic activity is major contributor to the high frequency components (HF), while the low frequency components (LF) are mediated by both the sympathetic and parasympathetic modulations. The ratio of low frequency to high frequency (LF/HF) was then calculated which is an indicator of the sympatho/vagal balance.

EIA was assessed using self-reported pain measures, ie. visual analogue scales for pain (muscle and/or joint pain) and headache intensity and the SF-36 bodily pain subscale. These were presented to participants prior to and following the exercise test and the autonomic measures.

Results

CFS/ME patients reported experiencing the exercise test as “very hard” and the healthy controls as “somewhat hard,” but all of the cardio-respiratory measures were similar, showing that the exercise was carried out in a standard way and that both groups have the same exercise capacity.

The aim of the experiment was to discover whether autonomic-mediated recovery following exercise is associated with the magnitude of EIA. Impaired heart rate recovery following exercise was not associated with lack of EIA; however a change in headache severity, as a type of EIA, was related to the reduced parasympathetic reactivation during exercise recovery. This implies that the efficacy of autonomic recovery can possibly mediate the degree of EIA in patients with CFS/ME.

The results showed that diastolic BP correlated with larger exercise-induced reductions in pain severity in both healthy controls and those with CFS/ME. Poor recovery in diastolic BP indicates that BP is still elevated 10 minutes after exercise and has not returned to pre-exercise levels. When BP increases there is more blood in the arteries, stretching these arteries, which in turn increases the activity of the baroreceptors. Hence, the study’s findings indirectly suggest the involvement of arterial baroreceptors in EIA. Previous research has shown that baroreceptor reflex sensitivity has an important mediating role between BP and pain.

In the study, poor recovery of LF in people with CFS/ME was linked to lack of EIA of headache. As previous research has showed that the baroreflex is responsible for a part of the LF component of HRC, again the current finding suggests that the arterial baroreceptors have a role in the mechanism of EIA.

Discussion

Reduced baroreceptor reflex sensitivity has been suggested in CFS/ME and other chronic pain conditions. Oxidative stress has been found to modulate baroreceptor sensitivity and it has been observed that post-exertional oxidative stress responses occur earlier and lasts longer in CFS/ME. It is a possibility that drugs affecting oxidative stress production could influence reduced baroreceptor reflex sensitivity in these patients.

Furthermore, it has been shown that physical training causes an increase in parasympathetic tone in healthy people, so this may help to improve autonomic balance in people with CFS/ME. At present it is not known what the training intensity in CFS/ME would need to be to improve parasympathetic tone in these patients, as care needs to be taken not to exacerbate symptoms.

When interpreting the findings and possible implications of the current study, limitations should be taken into account. These include the use of self-reported measures to assess pain (thresholds/tolerance) when more objective measures would be more reliable, and continuous BP monitoring could be used instead of the non-invasive technique. Furthermore, the cross-sectional study design does not allow conclusions to be drawn about causality.

Conclusion

Reduced parasympathetic reactivation during recovery from exercise is associated with the dysfunctional EIA in CFS/ME. In addition, poor recovery of diastolic BP in response to exercise, with BP remaining elevated, is associated with reductions of pain following exercise in CFS/ME, suggesting a role for the arterial baroreceptors in explaining dysfunctional EIA in CFS/ME.

Unravelling the mechanisms responsible for the dysfunctional EIA in response to exercise in people with CFS/ME is likely to be a crucial step towards treatment. Potentially, drug treatments in combination with graded exercise therapy could improve baroreceptor reflex sensitivity and parasympathetic tone during exercise recovery in CFS/ME patients, which in turn may improve EIA.

Disclosure: This study was funded by the Ramsay Research Fund of the ME Association (United Kingdom). The manuscript of this study is currently in press with Pain Physician.

Key point summary:

- The first step to effective research is to listen to patients.
- Quantification is key, preferably using universal, standard tools that are approved by the European Medicines Agency and US Food and Drug Administration
- Data equals power and using large cohorts is essential as it enables stratification
- Do different types of science and be prepared to think outside the box.

Prof Jones explained that he would be talking about research that he and his team had been undertaking for a number of years, and highlighting lessons learned that might be useful for the field of CFS/ME research.

His research is focused a fatiguing liver condition called Primary Biliary Cholangitis (PBC), previously known as Primary Biliary Cirrhosis. The name was changed at the request of the patient community, with the support of clinicians and researchers— “A really interesting story of patient empowerment,” commented Prof Jones.

Listen to patients

Reviewing what he had learnt over the course of his work with PBC, Prof Jones said that the first thing he had learned was to “listen to patients, and keep listening.”

For at least the first 150 years of the disease, symptoms of PBC were very much what you would expect to see in someone with liver disease, eg. jaundice, encephalopathy. But it has since been accepted that other symptoms of the condition are more common, namely fatigue and cognitive impairment that patients worry might be dementia. “We have changed the model for what this disease is, and persuaded the rest of the world to do the same.”

Prof Jones reflected on the original work in this area by Thomas Addison, describing him as the “father of auto-immunity,” before presenting work by a medical student Jane Goldblatt in 2003. It was Jane that first observed that PBC patients were all fatigued at the end of their appointments, something clinicians had not really registered.

“If you want to make a difference in this field, get medical students interested early,” interjected Prof Jones at this point. “They bring energy and vision, and you can make a difference very quickly.”

As part of her integrated degree project, Jane undertook a geographically based cohort study with PBC patients and case controls from the community to measure their fatigue. This study has now been cited hundreds of times, with PBC patients being significantly being more fatigued than healthy controls.

Prof Jones then moved onto to talk about work he has undertaken with Prof Julia Newton, looking at fatigue and activity levels in patients with PBC. One study, for example, found that the severity of fatigue perceived by patients was accurately reflected in their reduced level of physical activity.

In conducting his research, Prof Jones has found that words are equally as powerful as numbers when it comes to convincing people. He cited a patient called Tilly, who shared her experience with the research team to offer rebuttal to a funder that suggested that fatigue alone was not a symptom serious enough to warrant treatment. As a result, the funder was persuaded and a research award was granted.

Tilly said: “The problem with fatigue is that it is hidden. I don’t look different from other people: when I say I am tired they tell me how tired they are, and if I try to explain the difference they do not understand what I am talking about. When I was first diagnosed my GP told me that I would never get any sympathy as I would always look reasonably well and my symptoms would have no impact on other people’s understanding of my life. The fatigue that I and my fellow PBC patients contend with is mind numbing. You feel as if you are in a fog, you can hardly lift one foot in front of the other, everything is so difficult. You go shopping and then cannot unpack the groceries, so the frozen food defrosts and has to be thrown out.”

Quantification is key

Prof Jones stated that his second key learning point was that quantification is key. Measuring is critical, as is developing universal, standard tools that are approved by the European Medicines Agency and US Food and Drug Administration: “Probably the most important thing that we did is the development of the PBC-40.”

This is a patient-derived, disease-specific quality of life measure developed and validated for use in PBC, funded by the National Lottery. It this has become the tool that all researchers employ, being free to use to anyone who wants it.

The PBC-40 has a variety of domains. Fatigue is the major factor in the experience of patients, but it is complicated by cognitive, social and emotional dysfunction. Social isolation symptoms are also very important.

The power of data

Data equals power and cohorts are key, said Prof Jones as his third point. From starting out with 100-person studies, he and his team are just about to pull together a study of 10,000 people with DBC.

A commitment to developing very large cohorts, phenotyping them and keeping them going, gives us an unparalleled level of data, he said, adding that this plus the universal tool in the form of the PBC-40 is what has “cracked this field open.”

By having such a large cohort, you can look at the inter-relationship of symptoms. Showing the results of some recent work, Prof Jones explained that, if you take people’s perception

of quality of life with PBC and ask them what factors are important, the answers are fatigue, anxiety, depression, social isolation.

“This last one is really important, and along with fatigue and cognitive symptoms, it’s the one I didn’t spot when I was seeing patients,” he said. But it determines how much the symptom of fatigue has an effect – so social support can transform fatigue into a non-problem.

Disease models

Point number four: If fatigue doesn’t fit the fatigue model, it may be the disease model that’s wrong.

“In CFS/ME I see that there is considerable debate over the disease model but I think this is looking at it the wrong way round,” said Prof Jones. “Go where the answer is – don’t hammer a square peg into a round hole.”

He pointed out that there is no link whatsoever between how bad your symptoms are and how severe your level of liver disease. This leads to a therapeutic issue, whereby you can have people being treated by and responding to a first-line therapy called ursodeoxycholic acid, and people not being treated by (and therefore not responding to) ursodeoxycholic acid – and their level of fatigue is identical.

Nor does a liver transplant helps – transplanted patients and non-transplanted patients also have the same severity of fatigue. Finally, referring to his August 2016 paper in the New England Journal of Medicine, Prof Jones highlighted that a game-changing drug for the disease, Obeticholic Acid, has no effect on symptoms either.

Stratification

By using very large cohorts of patients, you can investigate the complexity of a condition through stratification. Prof Jones receives funding for this from the MRC, which allows him to take thousands of patients and split them into groups to understand the biology of PBC.

A classic example of this, he says, is the discovery that PBC is more of a problem in young people – this was completely unknown until a significantly large cohort was studied.

This all leads to a key question, said Prof Jones: is fatigue in PBC a brain thing or a peripheral thing? The answer is, of course, that it’s both – but unless you stratify into different sub-groups, you will get terribly confused about this.

Referring to work undertaken by the UK PCB consortium (www.uk-pbc.com), he showed how they had split patients into those with severe and those with mild/moderate fatigue, looking for three features to identify underpinning symptoms: autonomic dysfunction, sleep disturbance and depression.

Prof Jones then outlined the steps they had taken to target treatments for the patients experiencing each combination of features. He noted that the reason that men were less

fatigued than women is because they have less autonomic dysfunction, and there appears to be a direct link between the two.

Dense white matter lesions show an organic change in people with PBC – which would explain why people do not get better even after a liver transplant. Prof Jones presented some work undertaken by him, Julia Newton and Clare McDonald, which shows that brain function is still abnormal post-transplant. Looking at the symptom of cognitive impairment, organic testing has shown that the level of impairment relates to the number of lesions.

Different types of science

Prof Jones' final piece of advice to CMRC delegates was to "do different types of science – in my career, going completely left field has made such a difference."

He described using functional MR scans on muscles to detect pH changes, showing that acid production is seriously increased in people with PBC – so when they feel like they have run a marathon, they are actually describing it pretty accurately. A similar thing occurs in people with CFS/ME, along with taking much longer to recover, and again he referred to the links here with autonomic dysfunction.

Prof Jones also urged careful planning when it comes to trial design. "We have become trialists because that's the way you get treatments into practice," he said. "You need evidence that interventions work."

He emphasised the importance of having a dialogue very early in the process with regulatory bodies, to make sure you get the evidence you want. By way of illustration, he highlighted a recent trial of Rituximab for fatigue in PBC.

This trial recruited 55 patients into a single centre trial, with patient input into the design of the trial. It's the first trial ever done in liver disease where fatigue is the primary end point – "It says to the world that fatigue is important enough to treat." The protocol has been published so that other researchers could use it to do similar trials in the future, and findings are currently being analysed.

But what about the brain? A study using MR scans has shown that there are areas of the brain that are abnormal in PBC patients compared to controls, and what's interesting is that all these patients are within six months of disease onset. This shows instantly why none of the treatments described on the previous page are effective, and means the treatment paradigm must be changed if patients are to experience any improvement.

Coming back to trials, Prof Jones explained that he had been in touch with the FDA, who were "very sympathetic and supportive" of a treatment trial for PBC, and have asked for patient impact data and mechanistic data are both important here.

Using animal models, Prof Jones outlined work undertaken to see if drugs could be used more effectively, and concluded that there is a window of opportunity for PBC patients to take medication after the disease has started but before the onset of symptoms of fatigue. He and his team will now do an early intervention trial in younger patients at high risk of fatigue using MR as an early response indicator.

What does this tell us?

In conclusion, Prof Jones said that, going back to basics, we are dealing with problems of energy. “Sometimes, we overcomplicate fatigue,” he suggested. Is the common link between peripheral and central fatigue just energy? Would manipulating the energy balance through bioacids treat fatigue generically? Could we try using obeticholic acid in CFS/ME as we are doing in PBC to see if it reduces fatigue?

“The answer to all these questions is that you have to be bold, and you have to get industry-interest in supporting these things, but ultimately they will go after real opportunities,” he said, before reiterating the importance of listening to patients.

“I started my career not believing that fatigue is real in PBC,” he said. “Scientists look at evidence, and I said that I never saw it in my patients. But that’s because I wasn’t asking my patients – and clinical data clearly shows that fatigue is the same across all patients, regardless of whether you ask them or not.”

He concluded his presentation with a quotation from US president John F Kennedy: “We choose to go to the moon in this decade and do the other things, not because they are easy, but because they are hard, because that goal will serve to organize and measure the best of our energies and skills, because that challenge is one that we are willing to accept, one we are unwilling to postpone, and one which we intend to win.”

By a series of steps, with mistakes along with way, we are making progress, said Prof Jones, and through this I truly believe that we can change the lives of people with PBC. By uniting under a common purpose, CFS/ME researchers can do the same.

Closing remarks and reflections: Where next for the UK CFS/ME Research Collaborative?
Prof Esther Crawley, University of Bristol

Following presentation of the poster prize to Alice Russell for what *Prof Hugh Perry* described as her stand-out piece of work, Prof Crawley thanked all the delegates for coming, and gave a brief summary of presentations over the two days.

Outlining next steps, Prof Crawley talked of the appetite for the MEGA study, and wanting to use genetics and new methods to understand CFS/ME. “MEGA will be a resource for all researchers,” she confirmed.

CONFERENCE FEEDBACK

The following is verbatim feedback gathered from Professional, Student and Associate Members after the conference using a simple questionnaire.

How would you rate the conference overall?

- 67% said very good
- 28% said quite good
- 5% said average.

How relevant was the conference for you and your personal/professional interests?

- 50% said very relevant
- 39% said quite relevant
- 6% said average
- 5% said not very relevant.

Three delegates commented:

- As a newcomer to ME / carer of a 26 yr old daughter, I found it quite informative and nice to put faces to some names on studies.
- Day 2 was more relevant than the first day, but of course not all topics do speak to one's interest. Some oral presentations were quite short in comparison with others, and sometimes there was no time for questions. I would suggest plenary speakers to have longer talks but oral presenters to have equally long, but shorter talks (+/- 20 mins) and at the end of each session, the ability to ask questions (+/- 10 mins) to all the presenters from that session. Less information on numbers (cases, spending) more about what is known & what / which action we can still undertake.
- Relevant in that the day exposed how little, despite the thousands of £s of funding the researchers have achieved, surprisingly little.

For Professional and Student Members: what value do you feel participation in the conference will add/has added to you in your role?

- Experiencing top level, cutting edge research has added greatly to my ideas and hypothesis on ME. The Networking opportunities have been instrumental in identifying potential sponsors for a PhD project.
- As a clinician, knowing the volume and variety of research going on help to maintain enthusiasm and optimism. I can use a lot of the information to cascade to colleagues both CFS/ME and primary care and add to the explanations I use with patients to make sense of their symptoms.
- A better understanding of where my research fits in the bigger picture.
- Gained a more rounded knowledge of what's going on. Chance to meet with fellow members
- New ideas for future studies and collaborations. Opportunities for networking.
- Connections and inspiration
- Feeds into thinking about developing ideas and models. Networking
- Improved my understanding of CFS/ME from a clinicians aspect ie. management / research and patient perspective.

- Understand of where research might be heading. Disappointing lack of presentations on diagnosis and management. Equally trials of therapy.
- Each CMRC conference has felt like a step forward for the field of CFS research, esp in bringing new researchers to the field.
- The huge plethora of topics covered at the conference help to show the complexity of the condition and how it is treated. It also shows the way different research groups target different facets of the same condition. Networking & meeting others in the field is very interesting and could lead to further collaborations.
- Dissemination of study results
- Opportunity to engage with others

What plenary sessions, presentations and workshops would you like to see at next year's CMRC conference?

- Someone with ME to talk about their story or a session by those with CFS/ME
- Exactly what I saw this year, just with a focus on updates and possible treatment ideas. The feeling I get from patients I have spoken to is that the data is just data, and not a solution, and does not constitute progression in the patients' eyes.
- Some work on Stratification (Leonard Jason?) and update on Rituximab
- Support for parents / family of patients.
- All of the ME patients that I have met in the year my daughter has been ill felt alienated, distanced, ridiculed, humiliated, frustrated. An easy to comprehend, accessible update for those non-scientists and more of a welcome at such conferences.
- Review of why CBT/GET downgraded in USA & why we aren't doing the same in the UK.
- Invite speakers with expertise in other types of chronic fatigue: post-cancer, post-trauma also, fibromyalgia.
- Information about MEGA. Further work into PoTS
- Something regarding pain in CFS/ME (plenary / presentation). Workshop regarding pain assessments for researchers on the one hand and for clinicians on the other hand. Something regarding cognitive aspects in CFS/ME (plenary / presentation).
- Far more input from experienced / senior ME patients, given the lack of understanding / empathy of so-called experts. presentation by senior ME patients to try & decrease the ignorance of the medical profession & researchers.
- More small funders / researchers.

To find out more about the CMRC, including how to become an Associate, Student or Professional Member, visit www.actionforme.org.uk/CMRC

