Research Update: Metabolomics and ME/CFS – Dr Morten and the Oxford research centre 13th August 2018

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Introduction

The ME Association has been working for some time with Dr Karl Morten and the research team at Oxford who have recently been honing their approach to the study of metabolomics in ME/CFS.

Before embarking on a new study utilising samples from the ME/CFS Biobank for the ME Association, they have been conducting research using samples from a patient cohort in Poland.

In this update, Jamie Strong, who has M.E. and is working with Dr Morten in one of the Oxford labs, provides a summary of how their approach to this important area of research has been developed.

But before we get to the update, Dr Charles Shepherd, Hon. Medical Adviser to the ME Association, provides a basic summary of what we mean by metabolomics and explains why it is so important to ME/CFS research.

Part 1

What do we mean by metabolomics? Dr Charles Shepherd

Why is the ME Association funding research in this area?

Metabolomics describes the way in which scientists study the metabolism – chemical reactions that take place at a cellular level and which are involved in everything the body does to maintain life.

By measuring the number and state of metabolites (small molecules) that are produced by our bodies during several metabolic processes, for example, as we convert food into energy, we can assess what is happening in both health and disease.

Metabolomics has become a very hot topic in ME/CFS because several research teams have determined it can provide further evidence that people with the disease are fundamentally different than healthy controls.

This would certainly make sense, based on what we know of the underlying disease process(es) in ME/CFS and the apparent lack of energy needed to perform functions that were previously enjoyed.

Metabolomics technology is 'large scale,' meaning that several thousand metabolites can now be measured, and more data recorded from a single sample of blood or urine.

More data will help us to:

- better determine what is going wrong in ME/CFS metabolism and if there are sub-groups that can be identified,
- identify diagnostic metabolic biomarkers, and, hopefully,
- point to treatments that can compensate for any defects in metabolism – the energy conversion process.

The metabolism is incredibly complex and can vary a lot even in healthy individuals, so it's important to collect as much data as possible from large numbers of patients and healthy controls.

This is why the ME Association Ramsay Research Fund is providing investment into metabolomics research at Oxford.

Dr Karl Morten is leading the project and is working with Dr James McCullagh, Associate Professor in Applied Mass Spectrometry at the University of Oxford.

The research will also try to replicate the findings from an important 2016 study by Dr Robert Naviaux that suggested ME/CFS could be caused by the body going into a state of semi-hibernation.

Funding has come largely from the 2016 ME Association Christmas Appeal. Total RRF investment £100,000.

Part 2
Metabolomics research at Oxford University by Jamie Strong

For this research we employ a technique called Mass Spectrometry to identify and quantify metabolites in the blood of ME/CFS patients and healthy controls.

Measuring all possible metabolites and comparing these between the patient and control groups, is an approach called metabolomics and it can be used to try and identify which areas of metabolism are affected in ME/CFS.

This approach has been used by others (Naviaux, Fluge and Yamano), who all published research in 2016 showing a difference in the *metabolite abundance profile* found in the blood of ME/CFS patients when compared to healthy controls.

In our research we have developed a multi-instrument approach which allows us to measure several thousand metabolites, including amino acids, energy metabolites, lipids and more soluble metabolites in a single blood sample.

The Polish Cohort

Our initial research has focused on a Polish cohort of 46 ME/CFS patients and 26 healthy controls provided by Dr Pawel Zalewski (Nicolas Copernicus University in Torun).

Dr Zalewski has worked previously with Professor Julia Newton (Newcastle University) on ME/CFS and dysautonomia.

ME/CFS patients were diagnosed according to the Fukuda criteria with a thorough clinical assessment of each patient.

Tests conducted include:

- a tilt table test to determine the functional status of the autonomic nervous system (ANS),
- measurement of cardiac output to determine the amount of blood delivered to various parts of the body,
- a body analyser using bio-impedance to determine basal metabolic rate (the amount of energy expenditure over time at rest), and,
- aortic stiffness which is commonly linked to an increased risk of heart disease and cognitive decline – measurement can help estimate an overall state of health.

Notably, post-exertional malaise was highly prevalent in this cohort, suggesting a detrimental response to exertion and to exercise in the majority of patients.

Following capture of sample data using the mass spectrometer, we then used statistical analyses (which allow us to work with large numbers of variables), and asked the question:

Are there differences in the ME/CFS samples which distinguish them from healthy controls?

Using two multivariate statistical approaches for modelling large datasets, we have been able to show that patients and controls fall into statistically significant selected metabolite groups; with some of the key differentiating metabolites including amino acids and lipids.

The two statistical approaches are principle component analysis (PCA) and orthogonal projections to latent structures discriminant analysis (OPLS-DA). These are able to determine the relationship of many thousands of metabolites between the control and ME/CFS groups.

Effect of exercise

We also studied the effect of an incremental exercise programme on a sub-group of patients and were able to examine how exercise affected the metabolic profile in blood. We found the exercise programme to be useful in simulating increased physical exertion and therefore as a means to isolate changes in metabolism that may result specifically from increased locomotor (movement) output.

The programme increased an individual's amount of physical activity in increments – a similar approach as Graded Exercise Therapy (GET) – but with the intensity of the prescribed exercise based on parameters such as VO2 max (the volume of oxygen used by the body during maximal exercise).

To measure this, subjects were asked to pedal on a stationary exercise bike (ergometer), until they could no longer sustain their maximal attained energetic output (measured in watts) by completing a test referred to as cardiopulmonary exercise testing (CPET). This provided a global indication of the integrated response to exercise of the pulmonary (lungs), cardiovascular (heart), haematopoietic (blood components), central nervous system (CNS) and skeletal muscle systems.

Comparison of the metabolic profile of ME/CFS plasma samples taken before and after exercise showed no significant differences between these time points. This suggested that exercise was unable to significantly alter the metabolome within the ME/CFS patient cohort.

Put simply, this indicates that the lesser-on-average amount of exercise/mobility experienced by patients is not likely a key mediator of the metabolic perturbations observed in ME/CFS.

Symptom severity and sub-groups

Whilst this multivariate statistical analysis allows us to separate controls from patients, it is not designed to determine correlations between individual metabolites and symptom severity. However, using a different statistical technique, linear correlation analyses, will give some indication of the importance of individual metabolites in potentially affecting symptom severity.

We are currently looking into this idea in more detail and one area involves comparing the metabolomes (profiles of metabolites) of patients allocated to groups based on their autonomic nervous system (ANS) functional capacity.

The ANS controls regulation of heart rate, blood pressure and orthostatic response amongst other physiological functions. To define these groups, subjects were designated as either being sympathetic (those with a higher heart rate and often cerebral blood flow insufficiency), or parasympathetic predominant (these subjects demonstrated absence of the sympathetic associated physiological response) based on a combination of physiological parameters measured during the tilt table test.

This approach may identify molecular pathways that show greater changes for example that are not apparent without the separation of patients into subgroups. Such subgrouping will help enable us to understand the potential importance of metabolic abnormalities to the manifestation of physiological abnormalities such as Orthostatic Intolerance (OI). This could potentially identify molecular pathways implicated in contributing to or causing such abnormalities in the body.

This approach may also help identify a potential subgroup of subjects falling under the Fukuda diagnostic criteria that don't respond deleteriously to Graded Exercise Therapy. Conversely, people could also be identified who show a deleterious physiological response to exercise which worsens their symptoms, indicating that they should refrain from engagement in exercise.

The metabolic profile of ME/CFS appears to be relatively consistent over time and retains the capacity to differentiate patients from controls following exposure to increased amounts of exercise. Further analysis will look at any changes in the most important metabolites as illness severity can fluctuate over time.

Through the analyses of physiological, clinical and metabolite correlations, we will essentially be conducting a pre-screen that prepares us for working with such a large data set. This will allow us to select interesting metabolite differences which will be examined in greater detail and may elucidate specific metabolites that fluctuate with severity of symptoms. This may lay the foundation towards developing a diagnostic blood test for ME/CFS.

Sub-group analysis

ME/CFS is likely to be not one distinct illness but comprised of a number of different conditions which lead to a common set of clinical symptoms.

It is therefore important, when working with a dataset of thousands of metabolites, to identify not only those that show the most significant differences as a whole, but also those with subtler differences that are potentially only evident in a sub-group.

This is difficult and time-consuming work, and the challenge is to recognise the significance(s) in sub-population changes which may not present a common change across all patient samples.

Driver of disease?

In our analyses, patients could be distinguished from controls by a small selection of metabolites.

To try and determine which of the statistically important metabolites could be potential causative drivers of the disease – rather than being a perpetuating factor that is simply part of a severe illness – we have been correlating levels of these compounds with clinical data.

We used a statistical technique called variable importance in projection (VIP) which determines the importance of individual metabolites in generating a model/capacity to differentiate two experimental groups.

For example, does a metabolite, shown to be elevated in ME/CFS, link strongly with orthostatic intolerance (i.e. the greater or lesser the abundance of metabolite, the worse the severity of an individual's OI)?

One of the strengths of the Polish cohort is the large amount of clinical data which was collected over a 3-hour consultation period with each patient. By combining clinical data with metabolomics, we have made some interesting observations when comparing the top 400 VIP metabolites.

Basal metabolic rate

Basal metabolic rate (BMR) is the rate of body energy expenditure over time. Initial comparisons indicate that basal metabolic rate is strongly correlated with two unidentified metabolites, that do not have accepted chemical names in the metabolomics database.

However, as variables such as age and BMI are well known to have an impact on basal metabolic rate we attempted to control for these variables whilst analysing males and females separately.

Males were found to have a higher average BMR and when correcting the correlation for age and BMI and using a form of regression analysis, these confounded the correlation with basal metabolic rate. Conversely, following these corrections, previously insignificant metabolites exhibit significant correlations.

Without correcting for confounding factors (age and BMI) none of the metabolites with high VIP scores correlated with fatigue scores. However, with confounder correction using partial regression analysis to account for these factors, one of the unknown metabolites showed a strong correlation.

Work in progress and the ME/CFS biobank

We are carefully analysing our data and identifying the best type of blood samples on which to carry out further metabolomics analyses.

This will be important when working on the large ME/CFS UK biobank cohort where we have a choice of plasma isolated using heparin and EDTA tubes. Our data has shown that the type of blood

collection tube, and sample processing, can have a significant effect on the metabolite profile identified.

The Polish cohort has been valuable on many levels, refining our approaches to sample collection and processing, comparing large amounts of data and identifying the importance of clinically well characterised ME/CFS patients.

Work on a new study funded by the ME Association Ramsay Research Fund is due to start later this month. It will examine 250 plasma samples from the UK ME/CFS biobank which includes severe, moderate and mild ME/CFS patients (fulfilling Fukuda and Canadian criteria) and two control groups, healthy controls and multiple sclerosis patients as a fatigue control group.

We will be sure to let you know when our work is ready for publication, and keep you updated as our new project gets underway.

Please help support the ME Association

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