New tools for the diagnosis and treatment of autism

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What is the problem?

Autism is a lifelong, neurodevelopmental disorder that develops in early childhood and affects how an individual interacts and communicates with other people. One in every 150 individuals has some form of autism. Individuals with autism are characterised as showing impairments in social communication and interaction and have repetitive, often ritualistic, patterns of behaviour. The precise cause of autism is unknown however genetic mutations have a key role.

At present, there is no cure for autism but treatments are available to those with the condition. Currently there is no medical test to diagnose autism, such as a blood test, for example. Instead, specially-trained doctors and psychologists evaluate a child's development and behaviour to make a diagnosis.

Recent research in our lab found different mutations in the EIF4E gene in patients with autism. While one patient had a break in the chromosome, four others had single nucleotide insertions in the chromosome region that controls EIF4E protein levels.

What are we interested in?

EIF4E protein is a key regulator of protein synthesis, particularly in the brain, where it is important for how neurones communicate to each other. Furthermore, mutations in other genes which can control the activity of EIF4E have been found in other autistic patients.

We want to find out what the effects are on neurones of increasing EIF4E. As EIF4E controls protein synthesis, we are looking for proteins which show either an increase or decrease when we increase EIF4E levels. These proteins may be potential biomarkers of autism. Also, we will look for compounds that can reverse the effects of increased EIF4E as a means to treat autism.

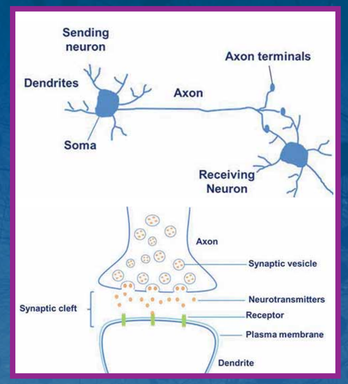

Figure 1: When one neurone sends a signal to another, it does this by releasing chemicals (neurotransmitters) which activate receptors on the receiving neurone. EIF4E is a key regulator of this process.

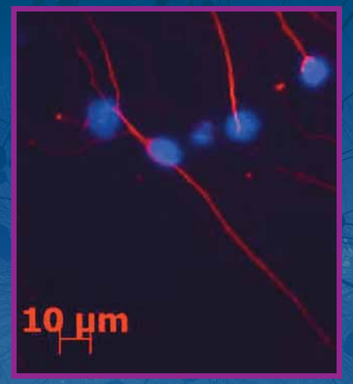

Figure 2: A primary neurone where the red highlights the whole neurone and the blue rpresents the nucleic acid.

How are we going to do it?

To investigate the effects of increased EIF4E on neurones we will isolate neurones and introduce additional EIF4E genes using a virus that is specifically built to produce EIF4E.

To look at the changes in protein content of the neurone when EIF4E is increased, we will be using protein gel electrophoresis, Western blotting, immunocytochemistry and advanced protein profiling techniques.

Western blotting is an analytical technique which identifies proteins in a cell extract on the basis of binding to specific antibodies. As we know the molecular weight of our protein of interest, we can use a size marker to identify the protein.

Immunocytochemistry is a technique which also uses antibodies for detecting proteins. We use a primary antibody which can bind to our target protein and then a secondary antibody which is fluorescent. This allows proteins to be visualised under a fluorescence microscope. This technique not only allows detection of the presence of a protein but also its location within the cell.

What did we find?

As we propose that only a small subset of proteins in the neurone will be changed when we overexpress EIF4E, there should be no significant change in total protein content of the neurone. To investigate this, we ran our neurone extracts on a 1D gel which separates out the proteins based on size and detected the proteins by using a protein stain (see Figure 3).

One method we plan to use is SILAC (Stable Isotope Labelling by Amino acids in Cell culture). This works on the principles of basic cell metabolism, whereby amino acids labelled with light or heavy isotopes are incorporated into newly-synthesised proteins. When light and heavy cell cultures are mixed, they remain distinguishable by mass, detected by mass spectrometry, and protein abundances are determined from the relative MS signal intensities (see Figure 4).

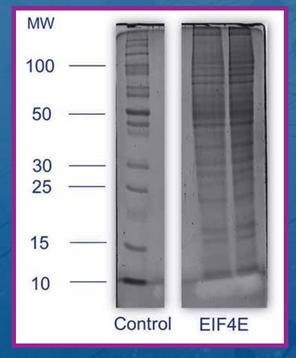

Figure 3: There was no overall change in protein levels between our control and EIF4E overexpressing samples, confirming our hypothesis that total protein content should remain the same.

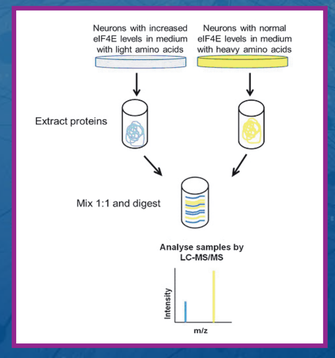

Figure 4: Neurons are grown in medium supplemented with light or heavy amino acids. Proteins are extracted, mixed, digested and analysed by mass spectrometry. Protein abundance of the two cultures is determined from MS intensity peaks of the peptides.

Who am I?

I am a PhD student at the University of Aberdeen. My project is about finding ways to understand more about, and point the way to developing treatments for, autism. I acknowledge the funding support from Medical Research Scotland and the help and encouragement of my supervisors.

Jihan Anderson started her PhD project, *"Identification and correction of the molecular and cellular defects in neurones caused by deregulation of the novel autism susceptibility gene EIF4E – an opportunity to develop treatments for autism"* in early 2013 and, while principally based at Aberdeen University supervised by Dr Berndt Mueller, she is also working closely with Dundee Cell Products Ltd.