CRISPR... a tool to crack genetic diseases

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What is the problem?

Personalised medicine is treatment that is tailored to an individual. How can we develop these?

Variations in people's DNA can make different individuals more or less susceptible to disease. If we can identify these variations we may be able to predict a person's likelihood of developing particular diseases and whether or not a patient will respond well to different treatments.

What are we interested in?

Genes are regions of DNA that provide the instructions to make proteins. They are made up of sequences that code for the protein as well as sequences that affect the gene's activity, which determine how much of, when and where a protein is produced. Regions that increase gene activity are called enhancers.

We are interested in a gene that encodes a protein that is found in the brain called the cannabinoid receptor-1 (CB_1 receptor). The CB_1 receptor is involved in the control of appetite, addiction and pain and changes in the activity of the CB_1 receptor gene may lead to conditions such as obesity or inflammatory pain. The CB_1 receptor is, therefore, a possible drug target for treating these conditions. Understanding what causes variations in the activity of this protein may advance such treatments.

We want to investigate whether a possible enhancer sequence of the CB_1 receptor gene affects the activity of the gene. We used a revolutionary new technique called the CRISPR/Cas9 system to investigate this. The CRISPR/Cas9 system allows us to make precise changes to DNA sequences in living cells, and even in animals, so we can observe the effects of these changes and discover the function of the DNA sequence which we have changed.

What did we do?

We used the CRISPR/Cas9 system to remove, or "knock-out", the enhancer of the CB_1 receptor gene in mice. We designed guide sequences which were injected with Cas9 into single cell mouse embryos. These sequences direct the Cas9 protein to the target regions of DNA. Cas9 acts like a pair of scissors to cut the DNA. In our case, the DNA was cut at two sites and the enhancer region of the CB_1 receptor gene between these two cut points was removed. The cell's own repair system then joined the free ends of the DNA together. The embryos were then implanted into host mice and the offspring were born with the enhancer of the CB_1 receptor gene "knocked-out" (see Figure 1).

We compared these knock-out mice to normal (wild type) mice to establish whether the enhancer has an important function.

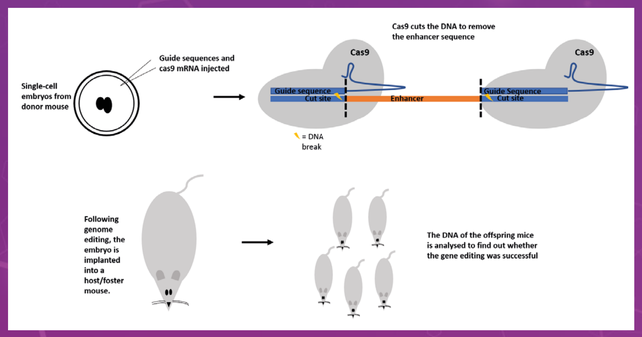

Figure 1 Following injection of guide sequences and Cas9 mRNA (a molecule related to DNA) into single-cell mouse embryos, the enhancer DNA sequence of the CB_1 receptor gene was "cut-out" by the Cas9 protein. Embryos were then implanted into host mice and the offspring mice were born with the enhancer knocked-out. Adapted from a figure in: Hay, E. A et al, Neuropeptides, 64, 19–25. Open access article available at http://doi.org/10.1016/j.npep.2016.11.010

What did we find?

By using a method called quantitative PCR, we could identify whether the activity of the gene had changed in the knock-out mice. We saw reduced levels of expression (activity) of the CB_1 receptor gene in the knock-out mice (see Figure 2).

To determine whether removing the gene's enhancer affected the function of the CB_1 receptor protein, we injected both knock-out and normal (wild type) mice with a compound (a drug) that is known to activate the CB_1 receptor. The normal response of mice treated with this compound is that they experience hypothermia (their core body temperature reduces), so we measured the core body temperature of the mice following injection. We found that the knock-out mice treated with the compound were less hypothermic than the normal (wild type) mice (see Figure 3).

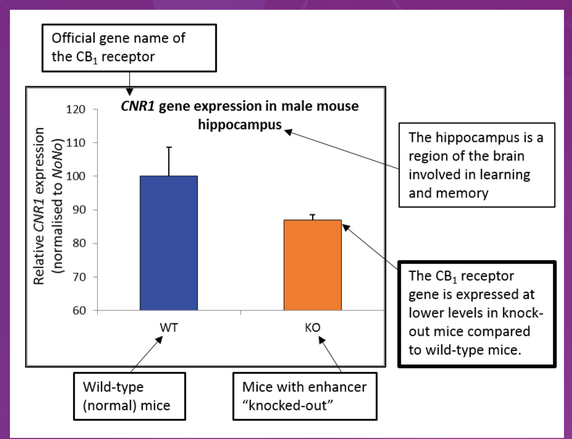

Figure 2 The activity of the CB_1 receptor gene is less in the mice with the enhancer 'knocked-out' compared to in normal mice.

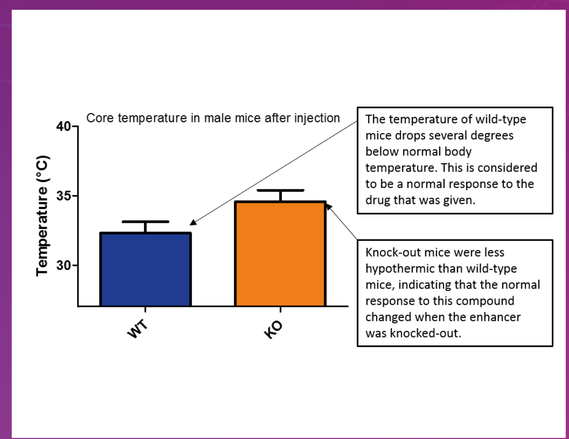

Figure 3 When the enhancer is 'knocked-out', the mice had an abnormal response to a compound (a drug) that activates the CB_1 receptor. This indicates that the enhancer has a role in controlling the normal function of the receptor.

What does this mean?

By using the CRISPR/Cas9 system in mice, we can alter specific DNA sequences and use these modified mice to identify the function of particular DNA sequences of interest. We have shown that an enhancer sequence in the CB_1 gene is important for the normal activity of the gene and that it has a role in controlling the normal function of the CB_1 receptor. By understanding the function of sequences, such as this enhancer, and variations within them, we may be able to predict whether or not someone will respond well to a particular drug and develop more 'personalised' medicines.

Who am I?

I graduated from the University of Aberdeen with a degree in Pharmacology in 2014. I am currently in the final year of my PhD at the University of Aberdeen, studying variations in DNA and what they mean. Doing a PhD has given me the opportunity to learn about and carry out research in this exciting field of biology and gain experience in using new techniques in the laboratory.

Elizabeth started her PhD project entitled *"The effects of genetic and epigenetic variation on the control of the cannabinoid-1 receptor gene and their role in disease and drug efficacy"* in 2014 and while principally based at the University of Aberdeen, supervised by Dr Alasdair MacKenzie, she is also working closely with GW Pharmaceuticals plc.