Investigating the role of sperm in the epigenetic inheritance of liver fibrosis

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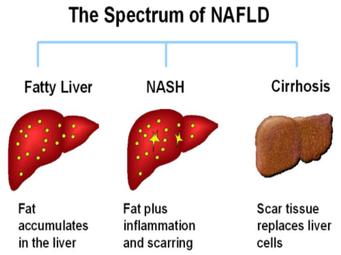
Objectives

- Use a range of techniques to investigate the characteristics of the sperm epigenome in mice.
- To identify epigenetic changes in the mouse genome which are linked to the inheritance of non-alcoholic fatty liver disease (NAFLD) in mice.

Introduction

- What is NAFLD? Non-alcoholic fatty liver disease is a chronic disease, involving the replacement of heathy, functioning liver tissue with scar tissue i.e. fibrosis. Progressive accumulation of scar tissue can lead to cirrhosis, however the development of cirrhosis from Non-alcoholic steatohepatitis (NASH) is unclear. This liver eventually becomes dysfunctional.



- Recent research has shown that the development of NAFLD can be regulated by epigenetic factors, such as DNA methylation, non-coding RNAs and histone modifications.
- The epigenetic factors cause changes to the gene expression without altering the DNA sequence, and can be inherited (1).

- As ~95% of histones are replaced by protamines in mice sperm, the aim was to identify which histones (and therefore nucleosomes) were retained, their location in the genome and which modifications they carry. Obtaining this information would then allow further understanding of the paternal inheritance of NAFLD in mice via epigenetic changes to the genome.

Methods

1. Western Blot

Western blot is a technique used to separate and identify specific proteins from a mixture of different proteins.

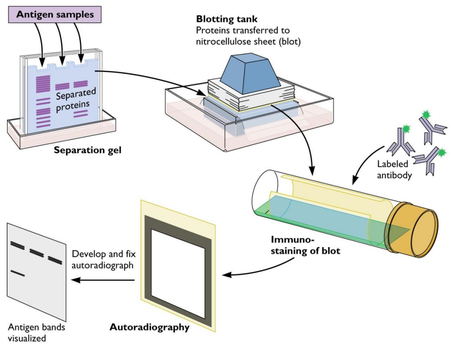


Figure 1.
A basic western blot protocol. Used to identify the types of histone protein present in the obtained sperm samples. Official protocol from Fibrosis Lab, Newcastle University.
Sperm samples were obtained from mice.

2. Sperm collection, Chromatin preparation, Mnase digest, DNA isolation, library preparation

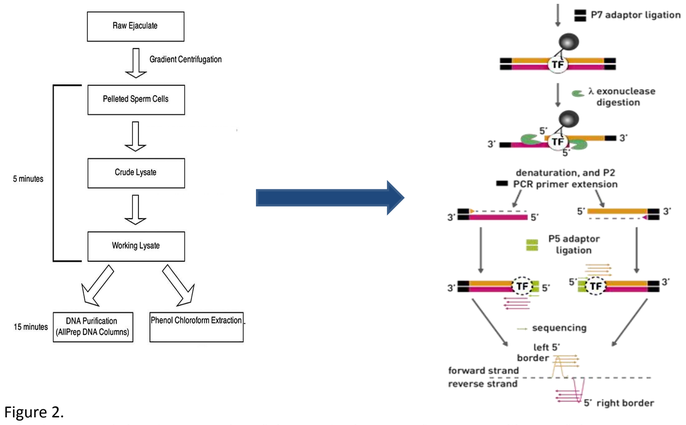


Figure 2.
A basic protocol showing examples of the required steps to form a DNA library of the mouse genome. Antione H F M Peters *et al*. protocol was used, with some alterations (2).

Sperm was collected from 5 mice, and the chromatin was extracted from the sperm. The chromatin was then pre-treated, before a micrococcal nuclease digest was carried out. The DNA was then isolated in order to make a DNA library. This library was then sequenced by next generation sequencing. Official protocols by Antione H F M Peters et al., 2013 and New England Biolabs.

Results

1. Western blot development

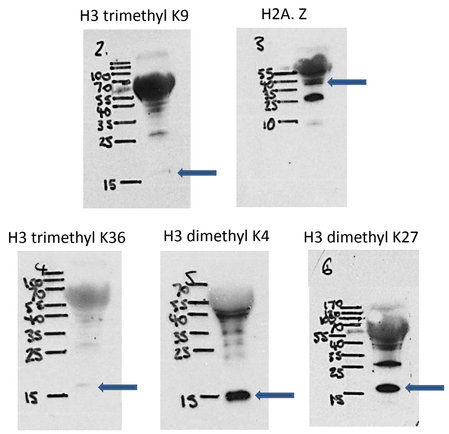


Figure 3.
Photograph of the western blot developments. Titles of each image refer to the specific anti-histone antibodies used against different types of methylation. The arrows highlight the protein band corresponding to the specific antibody used.
The large bands shown at ~55kDa could be the tails and other proteins contained within the sperm.
Antibodies against H2 trimethyl K4, total H3, H3 trimethyl K27 and H3 dimethyl K79 were also used, but did not show a positive result, i.e. did not appear to be present.

2. Bioanalysis of the DNA Library

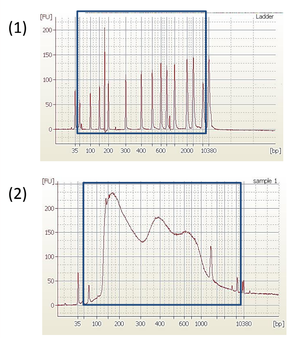


Figure 4.
Graphical representations of the size of the fragments produced by a bioanalyser after MNase digestion. (1) The DNA ladder. (2) The sperm sample. The area boxed highlights the size of the DNA fragments produced by the Mnase digest. (2) shows that a high concentration of DNA is present.

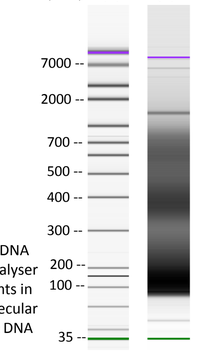


Figure 5.
Images of the different sizes of DNA fragments produced by a bioanalyser after MNase digestion. Fragments in each sample separated by molecular weight of the fragment. (1) The DNA ladder. (2) The sperm sample.

After the genome was mapped, nucleosomes were found to be retained at numerous positions throughout the genome. For example, the location of ten-eleven translocation (TET) and DNMT (DNA methyl transferase) genes were found; genes involved in methylation of the genome. This can lead to a change in gene expression and subsequently a NAFLD phenotype.

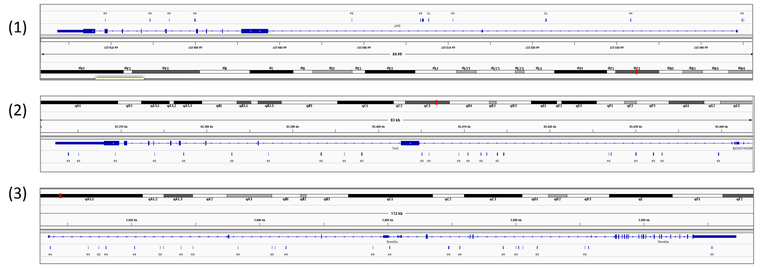


Figure 6.
Genomic locations of (1)TET2, (2) TET3 and (3) DNMT3a genes, produced by Interactive Genome Viewer genome browser.

Discussion, Conclusion and Future Prospects

- Main findings of this project involved the identification of the specific histone methylations that were present in the sperm of control mice.
- The DNA library enabled the identification of specific genes (shown above) involved in the histone methylation, which were present in the mice DNA.
- This is the beginning of a much larger 3 year project ongoing in the lab.

References
1. Derek A. Mann, Agata Page, 2015, Epigenetic regulation of liver fibrosis, Clinical and Research in Hepatology and Gastroenterology
2. Antione H F M Peters, Mizue Hisano, Serap Erkek, Sophie Dessus-Babus, Liliana Ramos, Michael B Stadler, 2013, Genome-wide chromatin analysis in mature mouse and human spermatozoa, Nature Protocols, 8, p2449-240

Acknowledgements
I would like to thank the Fibrosis Lab group for their support and guidance through this project.
I would also like to thank the Faculty of Medical Sciences for awarding me with a Research Scholarship, enabling me to carry out this project.