**////Title: Understanding Gene Mutations in Chronic Liver Disease**

**////Stand-first**:

Liver disease is reported to be the third largest cause of premature death in the UK, with 75% of patients being diagnosed too late for any meaningful intervention. Dr Matthew Hoare from the University of Cambridge, and Dr Peter Campbell from the Sanger Institute, lead a team conducting research into the genome changes associated with chronic liver disease to help understand the cause and consequence of these changes.

**////Body text:**

With the exception of the brain, the liver is the most complex organ in the body. It plays a vital role in keeping us healthy by controlling levels of sugars and fats in our blood, as well as clearing the blood of toxins. The most common types of cells in the liver are known as hepatocytes [he·pa·tow·sites], and these absorb nutrients and remove harmful substances from the blood.

Chronic liver disease has multiple causes including the long-term consumption of alcohol, viral hepatitis and non-alcoholic fatty liver disease, now thought to affect around 25% of the population. Dr Matthew Hoare, a cancer research specialist at the University of Cambridge and Dr Peter Campbell, a DNA sequencing expert from the Wellcome Sanger Institute, have conducted a large-scale study to assess the genome changes in cirrhosis caused by alcohol- and non-alcoholic fatty liver disease compared to healthy livers.

In an initial study, the team analysed the DNA from 482 liver sections from five healthy livers and nine livers with cirrhosis to look for changes in the DNA sequence, known as mutations, which may be associated with liver disease.

The liver is a particularly interesting organ, as in healthy people the main cell type in the liver – hepatocytes – are often genetically different from each other making identifying mutations in DNA very challenging. To overcome this challenge, the team took 1 cm2 segments from different areas of the livers and used a laser cutter to divide these into tiny sections made up of 100–500 hepatocytes.

This study highlighted the impact of chronic liver disease upon the genome and demonstrated that groups of hepatocytes in cirrhosis had an increased DNA mutation rate, with an average of 1,251 more mutations than those in normal livers. However, there were very few mutations within genes involved in HCC, that might explain why patients with cirrhosis are at risk of liver cancer.

Samples from healthy livers showed the expected pattern of having hepatocytes in the same area with different genetic make-up, known as polyclonality [poly·clone·ah·lity]. In comparison, livers from patients with chronic liver disease had significantly different structures, including nodules containing genetically identical hepatocytes separated by fibrous tissue, the hallmark of cirrhosis, showing that these nodules had often re-grown from a single cell after liver damage.

Dr Hoare, Dr Campbell and their team then built upon this study to further identify gene mutations under evolutionary selective pressure in chronic liver disease. They expanded the study by sequencing an additional 1,108 genomes from 20 liver samples. This gave a total dataset of 1,590 whole genomes from 34 livers, including five from individuals with healthy livers, 10 from patients with alcohol-related liver disease, and 19 from patients with non-alcoholic fatty liver disease.

The team identified a number of genes which were frequently mutated in several patients with unhealthy livers. One mutated gene in cirrhosis, called ACVR2A, has been previously associated with HCC – with approximately 10% of HCCs containing a mutated version of this gene. This suggests that ACVR2A mutations found in chronic liver disease might be involved in the development of liver cancer.

However, they also identified repeated mutations in three genes, all controlling major pathways involved in sugar or fat metabolism in the liver, that had not been linked with cirrhosis or HCC before this study.

FOXO1 [Fox-Oh-1] is a major gene involved in the control of sugar metabolism and is controlled by insulin. FOXO1 is active in the nucleus of hepatocytes and activates the pathways which break down fats and sugars. After eating, when insulin is present, FOXO1 moves out of the hepatocyte nucleus and stops activating these pathways.

From eight patients, the Cambridge team found 26 groups of liver cells, known as clones, which had all gained mutations in FOXO1. Six of these patients had hepatocyte clones which had all gained exactly the same mutation independently, and one patient even had nine independent clones with the same FOXO1 mutation in their liver.

This development of unrelated, but identical mutations, is known as convergent evolution and is powerful evidence that these FOXO1 mutations help hepatocytes to survive and thrive during the development of liver disease. When tested in liver cells in the laboratory, these FOXO1 mutations, all affecting one specific region of the protein, prevented FOXO1 from moving out of the nucleus in the presence of insulin, resulting in insulin resistance in liver cells with these mutations, showing that these mutations do lead to changes in sugar metabolism that potentially help the liver cell to survive.

CIDEB [Side-Bee] is another gene in which the team found a larger number of mutations than would be expected. CIDEB regulates the production of fat droplets within hepatocytes, with the mutations discovered preventing the formation and growth of these droplets. As with FOXO1, convergent evolution of mutations in CIDEB in several independent hepatocyte clones in the same liver was frequently seen, suggesting that these mutations might also improve the fitness of hepatocytes in disease.

Another significantly mutated gene was GPAM [Gee-Pam], which encodes an enzyme involved in the digestion of fats in hepatocytes. The team found fifteen different mutations in GPAM which impacted the function of the enzyme. Again, there was evidence for convergent evolution of these mutations, with one patient having seven separate clones with similar GPAM mutations.

The team were interested to discover if these mutations gave the hepatocytes carrying them a selective advantage – were they better able to survive and multiply than healthy cells? They found that clones with mutations in FOXO1, CIDEB and ACVR2A were larger than healthy clones, suggesting that these mutations do give a selective advantage and help the hepatocytes to grow better than nearby hepatocytes without these mutations. The team hypothesised that as all the mutations discussed affect genes involved in fat processing and storage, they may protect the hepatocytes by preventing high levels of fat from building up within the cells – a common feature of both alcohol-related and non-alcohol-related fatty liver disease.

This work by Dr Hoare and Dr Campbell is invaluable in helping us understand the genomic causes and consequences of chronic liver disease, cirrhosis and HCC. Future clinical studies will be able to expand on this work to understand whether these genome changes are an indicator of the future risk of liver cancer or cirrhosis and whether they can be used as a biomarker for liver disease progression or prognosis. Another exciting possibility will be to design drugs that harness or mimic the effect of these mutations to prevent the progression of liver disease.

This SciPod is a summary of the papers ‘Somatic mutations and clonal dynamics in healthy and cirrhotic human liver’, and ‘Convergent somatic mutations in metabolism genes in chronic liver disease’ both published in Nature. DOI: <https://doi.org/10.1038/s41586-019-1670-9> and DOI: <https://doi.org/10.1038/s41586-021-03974-6>

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