NEIL WEBB

DISEASE PROGRESSION

Divergent paths

Not everyone with a fatty liver goes on to develop more advanced disease. Understanding what triggers the progression could lead to better treatments.

BY SARAH DEWEERDT

Non-alcoholic fatty liver disease (NAFLD) is the most common type of chronic liver disease in developed nations, where it affects around 25% of the population. Its mildest form, steatosis, is characterized by the presence of large droplets of fat in the cells of the liver. At its most severe, NAFLD can lead to dangerous, life-threatening conditions such as liver failure or liver cancer.

There's a winnowing of those affected as NAFLD progresses. Up to 30% of people with fat droplets in their liver cells develop non-alcoholic steatohepatitis (NASH), in which the liver becomes inflamed. And about 20% of people with NASH will go on to develop scarring (fibrosis) of the liver, which is known as cirrhosis when it becomes severe enough to affect the liver's function, a condition that confers an increased risk of developing cancer.

Much of what is known about the course of NAFLD comes from paired-biopsy studies, in which researchers track the liver health of the same person through tissue samples that are removed years apart. At least a dozen of these studies have been conducted in the past 30 years. And, increasingly, such studies suggest that describing NAFLD as an orderly progression of stages oversimplifies the picture. For example, it's possible for a person with NAFLD to develop fibrosis without passing through the inflammatory stage of NASH, and for an individual with NASH to develop liver cancer despite the absence of fibrosis.

In some ways, this heterogeneity of progression should come as no surprise. Several other non-communicable chronic diseases, including cardiovascular disease, also show a variable course from person to person. So do many diseases of the liver, such as those caused by the viruses hepatitis B and hepatitis C.

However, the question of why NAFLD progresses in some people but not in others is an urgent one. "To me in the clinic, the biggest problem I have when I see patients is trying to predict those patients that are going to develop significant fibrosis and lead to morbidity," says Leon Adams, a hepatologist at the University of Western Australia in Perth.

Adams and other clinicians want to be able to give each patient more-accurate information about their prognosis. With effective drugs for NAFLD on the horizon (see page S86), clinicians will need to know in whom the condition is most likely to progress, to determine who should receive such medications. Although only a small fraction of people with steatosis will develop the end-stage complications of NAFLD, steatosis is so common that this fraction amounts to a large number of people. The high rate of incidence of steatosis also makes it equally important to know who doesn't need pharmacological therapies, to avoid burdening people with NAFLD — and health-care systems — with the financial cost and possible side effects of medicating those who are unlikely ever to develop advanced disease.

RATIONS OF RISK

NAFLD occurs most often in people who are obese (those with a body mass index of 30 or greater) and have metabolic syndrome — risk factors that contribute to cardiovascular disease and diabetes. These factors also influence the progression of NAFLD. People with more severe metabolic syndrome are also more likely to develop liver fibrosis. And when the factors worsen — an increase in body mass index, for instance, or development of the complications to metabolic syndrome such as diabetes or hypertension — fibrosis tends to worsen as well.

The progression of NAFLD is known to correlate with age. This may simply reflect the fact that the condition tends to evolve slowly; older people are more likely to have been exposed to risk factors for longer. But the pattern may change in coming decades, says Vlad Ratziu, a hepatologist at the Université Pierre et Marie Curie in Paris. "As obesity becomes more prevalent in the early years," he says, "the age of occurrence of the severe forms of the disease might also shift earlier."

Variation in how fat is stored in the body also influences the risk of NAFLD progression. A main feature of metabolic syndrome is the storage of fat in the abdomen; people who tend to carry extra weight in this part of the body are at greater risk than those who gain weight on the arms, legs and buttocks. People also differ in the tendency to accumulate stores of fat in the cells of the liver, rather than in fatty tissue.

Moreover, studies in mice suggest that the precise way in which fat is stored and processed in the liver influences the course of NAFLD. When stored as droplets of triglyceride inside liver cells, fat may pose relatively less risk to health. But other types of fat molecule, as well as by-products produced when fat is broken down and burnt for energy by cells, can be toxic to the liver. The presence of such molecules sets off inflammation, oxidative stress and injury to liver cells — all hallmarks of NAFLD progression.

The amount and type of fat that reaches the liver depends on a person's weight and metabolic status, and there is increasing evidence to implicate the collective microorganisms (microbiota) of the gut in the production of molecules that damage or trigger inflammation in the liver (S94). Genes are also a factor. "Genetic predisposition plays an important role in the progression from simple fat accumulation in the liver to fibrosis and the sequelae of NAFLD — cancer, fibrosis and inflammation," says Stefano Romeo, a molecular geneticist at the University of Gothenburg in Sweden.

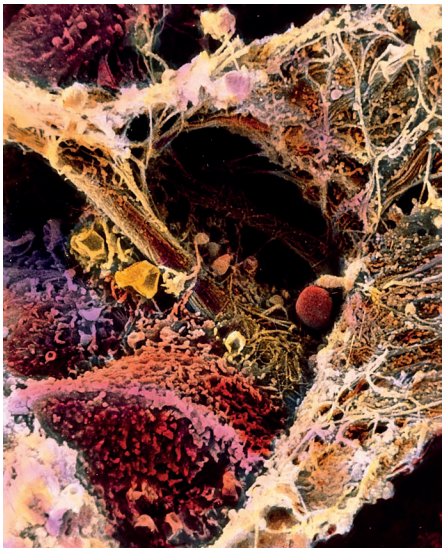
The strongest evidence points to the involvement of a gene called *PNPLA3*. In the United States, the form of *PNPLA3* that is linked to a high risk of developing NAFLD is most common in Hispanic people, the ethnic group with the highest risk of developing NAFLD, and least common in African Americans, who have a relatively low risk of developing the disease. *PNPLA3* encodes an enzyme that affects lipid metabolism in hepatocytes, the main cell type in the liver, which are responsible for many of the organ's functions. The gene also influences the activation of hepatic stellate cells, which build scar tissue and therefore contribute to fibrosis.

The finer details of the enzyme PNPLA3's function are still uncertain. But its influence on the epidemiology of NAFLD is clear. "It's been reliably and repeatedly shown to be a modifier of fat accumulation in the liver, of inflammation in the liver, of fibrosis in the liver and also of cancer," says Quentin Anstee, a hepatologist at Newcastle University, UK. "So it really is an independent modifier of each of those major steps in the pathogenesis of fatty liver disease."

Another gene, *TM6SF2*, is also thought to be involved in lipid metabolism, and researchers are trying to work out the precise influence it has on how and where fat is stored in the liver. The rarer form of the gene is associated with a greater risk of developing NAFLD but a lower risk of cardiovascular disease. On the flip side, the more common form confers a lower risk of developing liver disease yet a greater risk to the cardiovascular system.

That trade-off bolsters the idea that NAFLD is part of a broader condition — essentially, it is the liver-related consequence of metabolic syndrome. "*TM6SF2* is almost a determinant of which form of outcome of the metabolic syndrome you get," Anstee says.

Genetics accounts for only a proportion of the risk of NAFLD progression. Modifiable risk factors such as diet and metabolic condition are also major players. Yet advances in the understanding of NAFLD genetics are providing clues about which mechanisms to target when developing potential drug treatments. Eventually, Romeo says, "I think molecular genetics will help us to tease out how to treat these patients", enabling people with NAFLD to be sorted into groups on the basis of genetic variations that they carry.



P. M. MOTTA & T. FUJITA/SPL

Fibrosis (yellow) in tissue from the human liver.

WEIGHTY MATTERS

In the largest paired-biopsy study so far, about 40% of people show a worsening of NAFLD between biopsies, around 40% show no change, and roughly 20% show an improvement in, or the regression of, their condition (S. McPherson *et al. J. Hepatol.* **62,** 1148–1155; 2015). Regression tended to occur in those who lost weight or saw other improvements in their metabolic syndrome — achieving better control of diabetes or hypertension, for example.

Other studies that have followed people through lifestyle or dietary interventions reach a similar conclusion. "There's compelling evidence now that if you do lose weight through a combination of nutritional intervention and exercise, that you can improve not only NASH but also liver fibrosis," Adams says.

In an overweight person, shedding the pounds can have many effects, including a reduced level of insulin resistance, improved control of diabetes, curtailed release of inflammatory molecules from fatty tissue and a reduction in the amount of dietary fat that reaches the liver. "When you lose weight you improve a lot of things, not just the number on the scale," Ratziu says.

The regression of NAFLD is a testament to the extraordinary regenerative powers of the liver. Unsurprisingly, younger people and those with less severe disease are most likely to show an improvement. But beyond that, little is known about the mechanisms of regression or the factors that determine why one person shows an improvement but another does not.

That's because NAFLD regression is hard to study without repeated biopsy — only a small proportion of patients have undergone the invasive procedure more than once. Researchers are unsure whether those who participate in paired-biopsy studies, which are usually conducted at specialized hospitals, reflect the wider population of people with NAFLD.

Therefore, for a variety of reasons — to help determine the prognosis of newly diagnosed patients, to gain further insight into the nature of NAFLD, and to help monitor patients' responses to treatment, especially with potential drug therapies on the horizon — researchers are looking for non-invasive biological markers (biomarkers) of NAFLD progression.

So far, the search has been frustrating. A blood test developed to gauge liver fibrosis caused by viral hepatitis also seems to provide information about fibrosis in NAFLD. But it captures a relatively advanced phase of the condition — tracking the progression of NAFLD before fibrosis becomes established is trickier. Inflammation, the hallmark of NASH, has a similar biochemical footprint no matter where it occurs in the body, so it can be difficult to tie inflammatory markers in the blood to events that take place in the liver.

Enzymes known as alanine aminotransferases (ALTs) are produced by the liver in response to cell injury, and their levels in blood can be a good indicator of NAFLD progression and liver damage. But scientists have found that progression sometimes occurs in the absence of elevated ALTs. Normal levels of ALTs can't therefore be used to rule out active liver disease.

A more promising approach may come from epigenetics, in which chemical modifications on DNA alter the activity of genes without changing the DNA sequence. Anstee's group is looking at epigenetic marks consisting of methyl groups on DNA from genes expressed mainly in liver cells that circulates freely in blood. Other researchers are investigating small non-coding molecules of RNA called microRNAs that affect gene expression as possible biomarkers for NAFLD.

Innovative imaging approaches could shed light on NAFLD progression without the need for biopsy. Methods developed to measure fibrosis in people with viral hepatitis can also be used in NAFLD. And certain magnetic resonance imaging techniques show promise for quantifying fat in the liver, providing a window on the condition's earliest stage. "But where we're less good is distinguishing steatosis from NASH, or identifying subtle degrees of fibrosis in the liver," Anstee says. For now, the complex and variable course of NAFLD is still flummoxing those who try to tease it apart. ■

Sarah DeWeerdt *is a freelance writer in Seattle, Washington.*