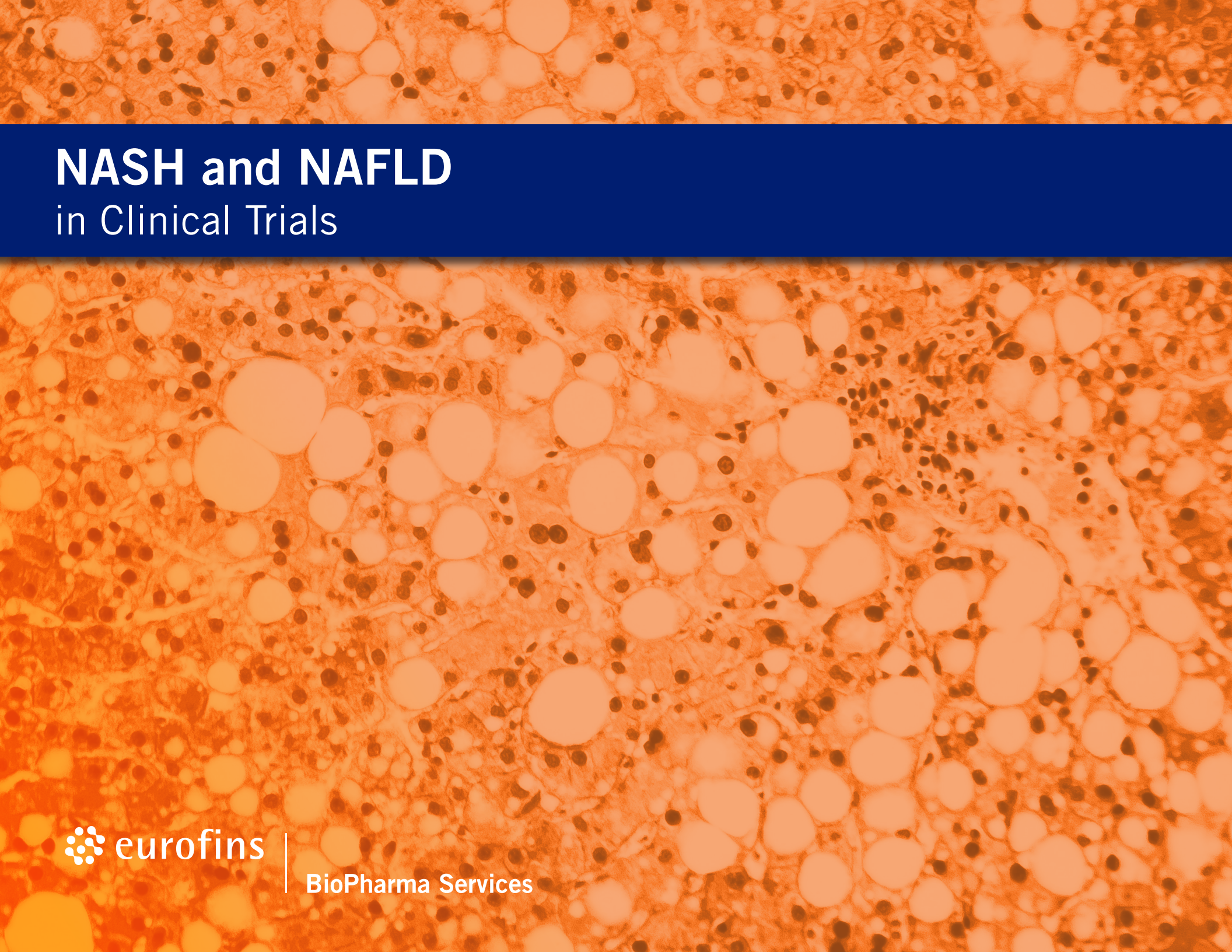
# NASH and NAFLD
## in Clinical Trials



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# Integrated Lab Performance and BioPharma Solutions From the Organization You Trust

Eurofins BioPharma Services provides seamless, end-to-end solutions to help clients progress through the drug development cycle through a single, experienced provider. Our integrated solutions deliver the most comprehensive range of state-of-the-art analytical technologies with an expansive geographic reach in order to support our clients' specialized testing needs and stringent quality and safety requirements around the world.

## Nonalcoholic Steatohepatitis (NASH) Testing

Hepatitis is an inflammation of the liver that is most commonly caused by viruses but may also be due to chemicals, drugs, alcohol, inherited diseases, or an autoimmune disease. One of the most common causes of chronic hepatitis is an accumulation of excess fat in the liver of people who do not drink excessive amounts of alcohol. This is also known as the fatty liver.

This condition develops gradually, usually over several years, with the intake of too many calories. Oftentimes, the first sign of the disease in an individual are abnormal results on routine blood tests. A liver biopsy may then be ordered in cases where the liver is enlarged and viral or if other causes of hepatitis have been ruled out. If the biopsy reveals that the examined liver tissue is excessively fatty, inflamed, and showing signs of damage, the condition is called Nonalcoholic Steatohepatitis (NASH). If a fatty liver is otherwise healthy and showing no signs of inflammation or scarring, the condition is called Non-Alcoholic Fatty Liver Disease (NAFLD). NASH can be severe, and can lead to cirrhosis in the liver. However, NAFLD usually causes no long-term harm.

NASH can progress to more serious disease stages such as advanced fibrosis, cirrhosis, liver failure, or liver cancer. Scientific articles also suggest that there is a higher risk of death from cardiovascular disease [1].

NASH clinical research is a crucial part of understanding this condition and to find treatments that slow down the progression of the disease and that create a better prognosis. Other factors like lifestyle, genetics, and demographic factors interact with the onset and progression of the disease. Each NASH clinical trial is a concrete step towards understanding those features and risk factors and discovering a true cure for NASH.

Eurofins BioPharma Services is a dedicated Laboratory CRO providing End-to-End analytical testing services in support of NASH clinical trials.

▼ [1] Reference European Association for the study of the liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidlines for the management of non-alcoholic fatty liver disease. *J Hepatol*, 2016;64(6):1388-1402

**Each of these conditions are most commonly seen in people with one or more of the following:**

- Metabolic syndrome
- Obesity, especially too much fat in the belly
- Hypertension
- High triglyceride levels
- Low HDL cholesterol
- Insulin resistance
- Type II Diabetes

# NASH and NAFLD in Clinical Trials

In Clinical Trials, Eurofins BioPharma Services offers comprehensive metabolic testing of up to 14 parameters to provide information about a subject's metabolism, health of their kidneys and liver, blood glucose and blood proteins.

Additionally, a dedicated liver panel is offered to detect, evaluate, and monitor liver disease or damage. The liver panel measures enzymes, proteins, and substances that are produced and excreted by the liver and may be affected by liver injury.

**Disease Definitions:**

- NASH: Overall hepatic injury including steatosis, inflammation, and/or ballooning.
- NAFLD: >=5% Macrovesicular steatosis

**Prevalence:**

- NAFLD: Impacting up to 27% population across a range of countries and ethnicities, strongly associated with obesity
- NASH: 20% of NAFLD population, 5% total

NASH is a progressive disease that worsens over time due to liver inflammation and the build-up of fat in the liver. Damage cannot be reversed. However, treatment and lifestyle changes can result in slowing the progression of the disease and create a better prognosis. There are still no approved therapies indicated for NASH patients. But that's about to change. Regulatory authorities and the scientific community have accelerated clinical trials and approval procedures for NASH, with the goal of providing patients with NASH access to approved therapy as soon as possible.

▼ [2] Reference The NASH Education Program

## HEALTHY LIVER

The liver is the largest solid organ in the body and it performs many essential functions, such as nutrient metabolism, protein synthesis, bile production, and glycogen storage. A healthy liver is blood-red with a smooth surface and contains 5% (or less) fat.[2]

## STEATOSIS

Fatty liver, or non-alcoholic hepatic steatosis, is observed in individuals who chronically consume excess calories and/or have a sedentary lifestyle, in the absence of significant alcohol consumption. Excess calories are stored in liver cells as lipids, resulting in a liver with fat content above 5% and a pale yellow color.[2]

## NASH

After enough excess fat has accumulated in the liver, chronic inflammation and cell death (ballooning) result in NASH. At this stage, patients have a higher risk of death from cardiovascular disease.[2]

## CIRRHOSIS

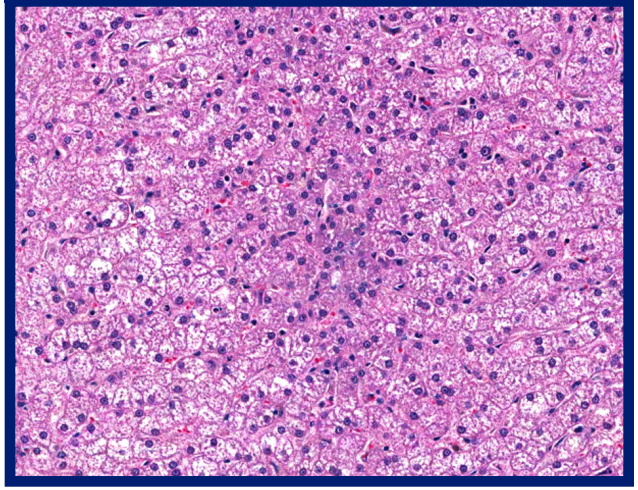
Chronic and continuous cell damage and ballooning result in the formation of fibrous scar tissue (fibrosis). Eventually, excessive scar formation will result in loss of liver function, a state known as cirrhosis or stage 4 fibrosis.[2]

## OUTCOMES

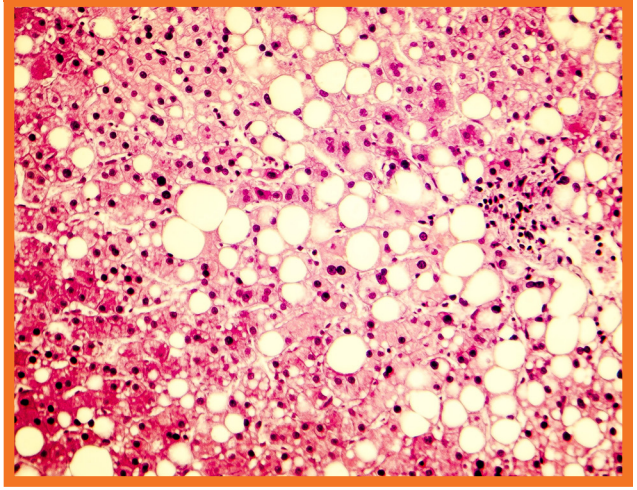
Patients with NASH-related cirrhosis are at higher risk of end-stage liver diseases, such as loss of liver function (decompensation), liver failure, and hepatocellular carcinoma (liver cancer). They are also at higher risk of death from cardiovascular disease and non-liver cancer.[2]

## Laboratory Considerations

### Normal Liver



### Nonalcholic Steatohepatitis



### Pathology: Liver Biopsy

A liver biopsy is associated with the definition of both NASH and NAFLD, where testing is required for FDA evaluations and efficacy claims.

### Biomarkers

Biomarkers are a rapidly evolving space that can add value to a trial by providing additional leading disease indicators beyond liver pathology.

### Calculations

Calculations are performed specifically with Biomarker test results. These calculations have clinical utility and their scores correlate with identification of disease state and severity.

### Clinical Genomics

Testing of genetic variations that influence the onset and or progression of NASH.

# PATHOLOGY SERVICES

A Liver biopsy is crucial for selecting patients with NASH for inclusion in clinical trials and, by implication, for treatment after new drugs are licensed. These clinical trials aim to include those patients considered to be at greatest risk of disease progression and liver-related morbidity and mortality. These factors are defined by the presence and the severity of liver fibrosis. Almost all drugs for NASH currently being assessed in clinical trials aim to treat the inflammatory activity associated with NASH, and therefore the population being considered for treatment are patients with active NASH and significant fibrosis.

Many patients screened for clinical trials undergo an invasive liver biopsy that does not show sufficient activity or fibrosis for inclusion. Under those circumstances, the patient could be considered to have undergone an unnecessary investigation that has not contributed positively to their care. Preventing unnecessary liver biopsy in patients with NASH is an important goal, both to reduce complications and increase the proportion of patients who enter trials or start treatments that aim to improve outcomes.

## Experience In Action

Eurofins BioPharma Services has been supporting many clinical trials with End-to-End testing solutions, including pathology services. We work with pathology experts that have participated in several clinical trials involving liver pathology including NASH, Viral Hepatitis, and Fibrosis/Cirrhosis.

Pathologist lead will recommend or consult on the decision making to ensure that an appropriate scoring system for fibrosis and inflammation is deployed, suited to meet specific protocol requirements.

## General Microscopic Detail

- Based upon review of routinely performed H&E and Trichrome stains.
- Exquisite histology with attention to section thickness and fixation is required for proper identification of microvesicular fatty change, Mallory hyaline and other subtle NASH findings.
- Consistent Trichrome staining for identification of early fibrosis findings.
- PAS, Reticulin and Iron stains may be added to the protocol if requested.
- Number of triads present in the sample.

## Non-Alcoholic Steatohepatitis Grading and Staging:

**Grade: 1, 2 or 3 (mild, moderate severe)**

**Stage: 1-4 (fibrosis, cirrhosis)**

Brunt EM, Janney CG, Di Bisceglie AM et al: Nonalcoholic steatohepatitis: a proposal for grading and staging of histological lesions. *Am J Gastroenterol* 1999;94:2467-2474.

## Granular Reporting

### NASH Clinical Research Network Scoring System

The activity score is determined by adding scores of steatosis, lobular inflammation and ballooning (0-8)

Kleiner DE et al: Design and validations of a histological scoring system for non-alcoholic fatty liver disease. Hepatology 2005;41:1313-1321.

# BIOMARKER TESTING

## Fit for Purpose

To support NASH clinical trials, laboratory biomarkers are Non Invasive Technologies, or NITs. These laboratory tests on blood samples are used to help improve diagnosis, staging of the disease, changes and prognosis, and can be divided into two separate categories. Safety laboratory markers are used to assess and monitor safety of the clinical trial subjects.

Biomarkers are also used to proof that the new treatment is effective: can we detect a therapeutic response to the treatment? These biomarkers can become surrogates for traditional clinical outcomes based on morbidity or mortality. Using efficacy biomarkers as surrogate endpoint can be very attractive as NASH typically takes years and years to build up. Therefore, it is important that an efficacy biomarker indeed is linked to the prognosis and outcome of the disease. Biomarkers are evolving rapidly and new ones are being discovered for both efficacy and safety applications.

Our biomarker validation procedures are fit-for-purpose and may range from CLIA validation to Advanced Validation of Biomarker Assays. For method validation of biomarker assays, a distinction will be made between biomarker data for exploratory purposes and biomarker data in support of efficacy/safety decisions for regulatory purposes. At Eurofins, we fully implement the published recommendations on biomarker assay validation.

### Safety Laboratory Markers

**Liver enzymes**

- AST [Aspartate amino transferase]
- ALT [Alanine amino transferase]
- GGT [Gamma-glutamyl transferase]

- Triglycerides
- Platelets
- HbA1c

### Efficacy Markers

- Enhanced Liver Fibrosis (ELF Panel) HA, TIMP-1, PIIINP [Recommended by NICE and EASLD for NAFLD Evaluation and Surveillance]
- CK18 M30 Aptoptosense [Caspase cleaved, measures apoptosis strong association with NAFLD]
- CK18 M65 Aptoptosense [“Total”, measures necrosis, strong association with NASH]

Further, Laboratory Biomarker test results can be used in Biomarker Calculations with a certain clinical utility. There are several calculation methodologies using laboratory result data. Calculations are performed in a formula or algorithm to support prognostic or diagnostic clinical utility. A wide range of calculations require planning at the beginning of the study to ensure that methods and context of calculations are tailored to the project and included in study set-up.

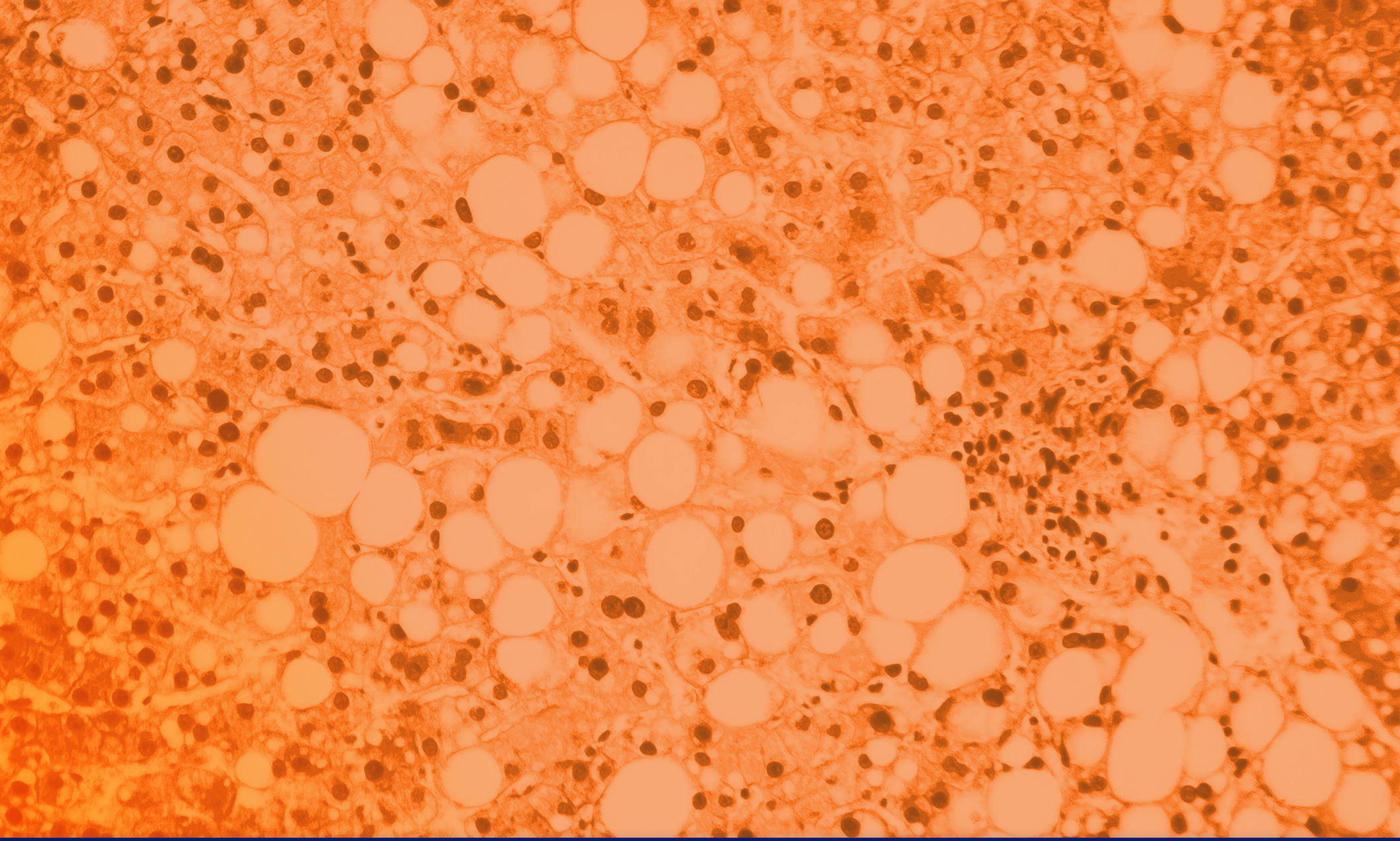
# CLINICAL GENOMICS

As our genetic make-up can influence the onset and progression of NASH, genetic testing can be performed to include or exclude, or group certain patients to a clinical trial. Some conditions may look very similar to NASH, but aren't actually NASH. Targeted genotyping can distinguish other liver conditions that have similar symptoms.

Prognostic Genetic Testing can be performed for previously validated genetic markers that can indicate expected patient groupings- this can include expected course of disease, risk of death, or other outcomes depending on the indication. Retrospective genetic testing is important to better understand responses during a clinical trial, generally reviewing a large number of genetic characteristics through Next Generation Sequencing, Whole Exome Sequencing, or a variety of other high-content analysis methods.

| Test Name | Type of Testing | SNPs Associated | Clinical Utility | General Usage |
|---|---|---|---|---|
| Wilson Disease (ATP7B) | Targeted Genotyping | R778L | Separate liver condition with similar symptoms | Exclusion Criteria |
| PNPLA3 | Targeted Genotyping or NGS Panel | Multiple including rs738409 | Risk factor for NASH, non-obese NAFLD | Retrospective Analysis |
| TM6SF2 | Targeted Genotyping or NGS Panel | rs58542926 | Risk factor for NASH | Retrospective Analysis |
| CETP | Targeted Genotyping or NGS Panel | rs12447924 and rs12597002 | Increased NAFLD prevalence | Retrospective Analysis |
| SREBF | Targeted Genotyping or NGS Panel | rs11868035 | Increased progression to NASH | Retrospective Analysis |

*Clin Mol Hepatol.* 2019;25 (1): 1-11. Publication Date (Web): 2018 August 08 (Review)



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