

PROVEN GLOBAL CONTRACT RESEARCH EXPERTISE
FROM DISCOVERY THROUGH CLINICAL SUPPORT

Drug Metabolizing Enzymes and Transporters in NASH

Research Application of Variants of Fatty Liver Disease (FLD) Tissues



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Aims and outline of the presentation

- Basic facts about fatty liver disease (FLD) and non-alcoholic fatty liver disease (NAFLD);
- Drug metabolizing enzymes and transporters in NASH;
- Introduction to the collection of normal and FLD tissues in the XenoTech Research Biobank

Fatty liver disease (FLD) and non-alcoholic fatty liver disease (NAFLD)

- Significant history of alcohol consumption differentiates these diagnosis.
- **Non-Alcoholic Fatty Liver Disease: NAFLD**
 - Includes the entire spectrum of fatty liver disease in patients who have no history of significant alcohol consumption;
 - Encompasses steatosis to steatohepatitis and steatohepatitis with cirrhosis;
 - The liver contains more than 5% fat by weight;
 - Presence of hepatic steatosis(fat) with no evidence of hepatocellular injury (no balloon degeneration of hepatocytes) and no fibrosis

Microvesicular steatosis

- Histologically no distortion of the nucleus
- Acute Fatty Liver of Pregnancy/HELLP
- Reye's Syndrome
- Nucleoside analogues, Tetracyclines, valproic acid
- Congenital Defects/Inborn Errors of Metabolism
 - LCAT deficiency
 - Wolman disease
 - Cholesterol ester storage disease

Non-alcoholic fatty liver disease (NAFLD)

Definition

- there is evidence of hepatic steatosis, either by imaging or by histology,
- there are no causes for secondary hepatic fat accumulation such as significant alcohol consumption, use of steatogenic medication, or hereditary disorders.

In the majority of patients, NAFLD is associated with metabolic risk factors such as **obesity, diabetes mellitus, dyslipidemia**.

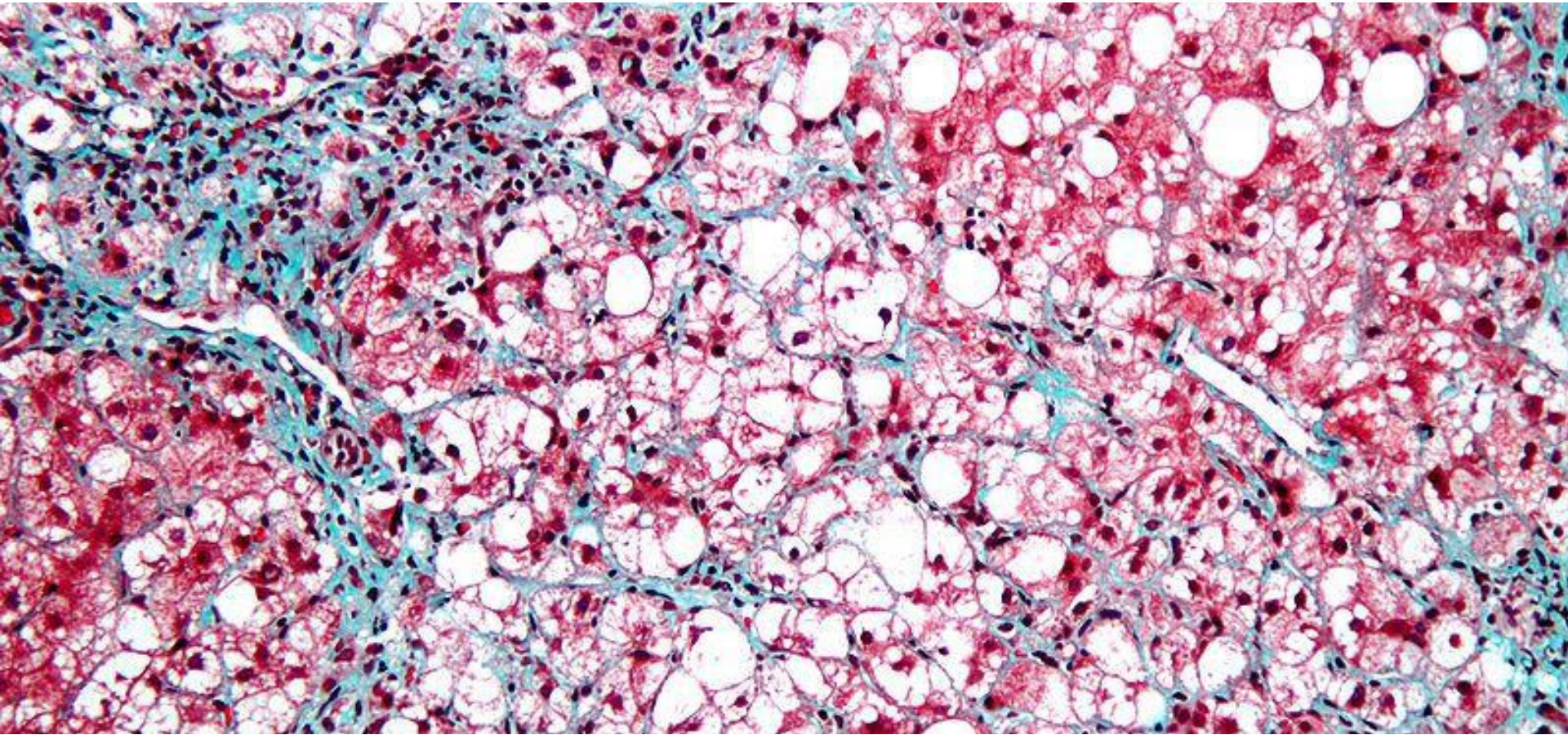
NAFLD is further histologically categorized into non-alcoholic fatty liver (NAFL), and non-alcoholic steatohepatitis (NASH).

NAFL is defined as the presence of hepatic steatosis with no evidence of hepatocellular injury in the form of ballooning of the hepatocytes.

NASH is defined as the presence of hepatic steatosis and inflammation with hepatocyte injury (ballooning) with or without fibrosis.

Am J Gastroenterol 2012; 107: 811– 826

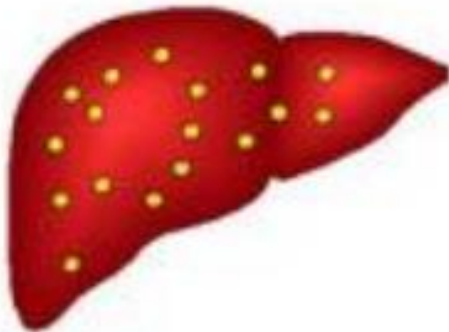
Steatohepatitis with fibrosis



NAFLD progression

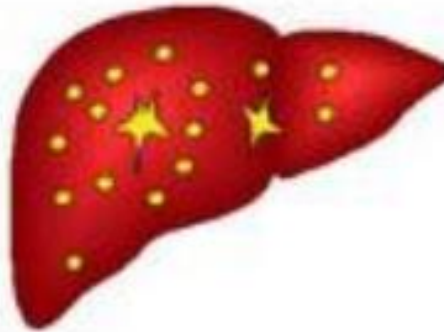
The Spectrum of NAFLD

Fatty Liver



Fat
accumulates
in the liver

NASH



Fat plus
inflammation
and scarring

Cirrhosis



Scar tissue
replaces liver
cells

Prevalance of NAFLD

Most common chronic liver disease in the US

(Adams LA. NAFLD. Ann Epidemiol 2007;17:863-869)

- Adolescent obesity has quadrupled in the past 10 years
- 1 in 10 pediatric patients in the US. NAFLD seen as early as age 2 with NASH cirrhosis seen at age 8!

In US

NAFLD: 10-46%

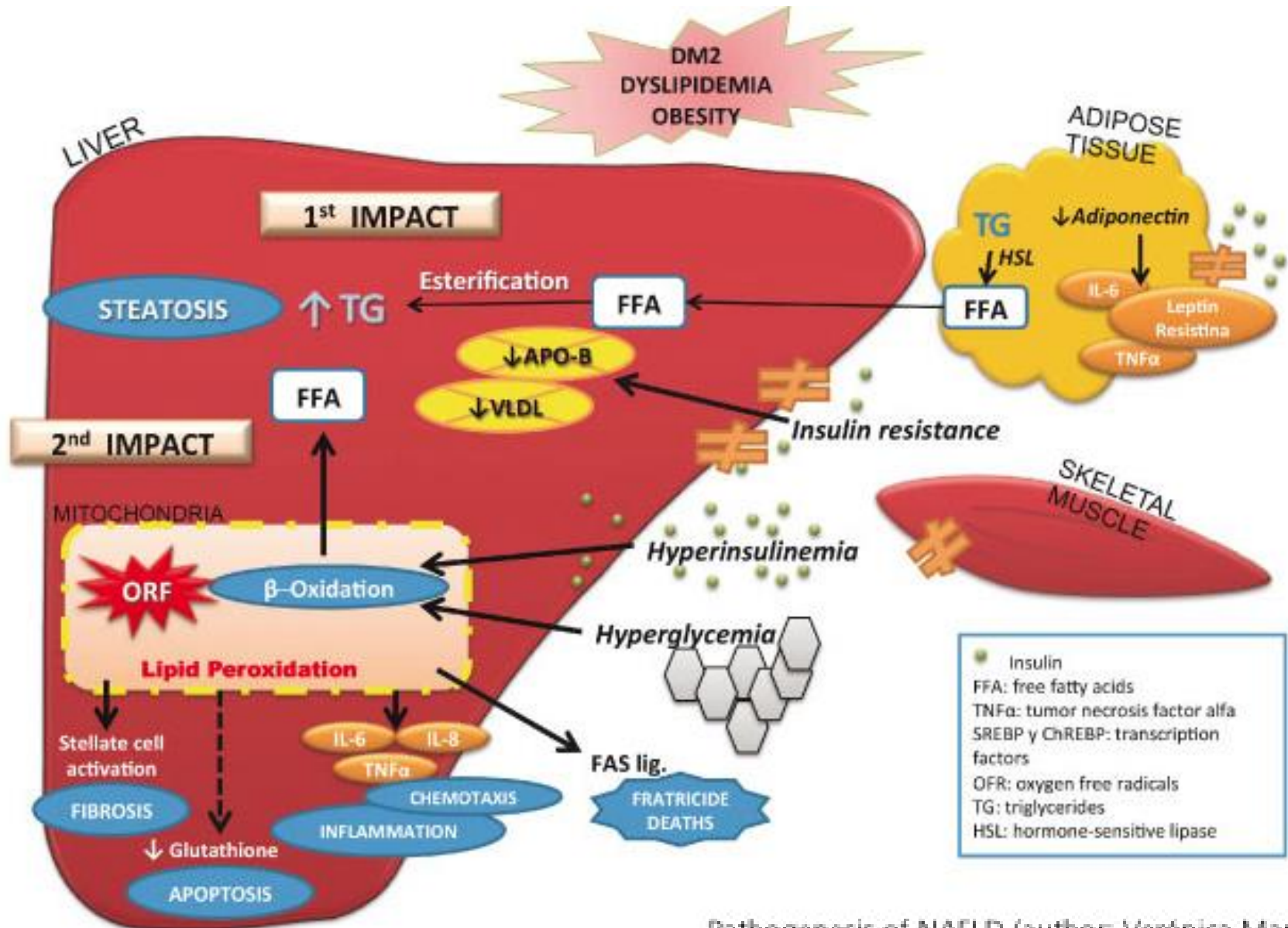
NASH: 3-5%

Worldwide

6-35%

- (Williams CD. Gastroenterology 2011;140:124-131 Vernon G. Aliment Pharmacol Ther 2011;34:274-285)

Pathogenesis of NAFLD



Drug metabolizing enzymes and transporters in NASH

- Drugs are being developed for multiple NASH targets. Given association of NASH with other conditions such as T2DM, dyslipidemia and obesity, these new drugs will be a part of poly-therapy therefore chances of DDI are high.
- Other imperative to study DME and drug transporters in NASH stems for a need for safe and effective dosing of current medications.
- The strongest evidence for clinically-significant dysregulation has been demonstrated for CYP2E1 and CYP3A4.

CYP2E1 in NASH patients

Chlorzoxazone clearance in biopsy-diagnosed NASH patients and matched controls.

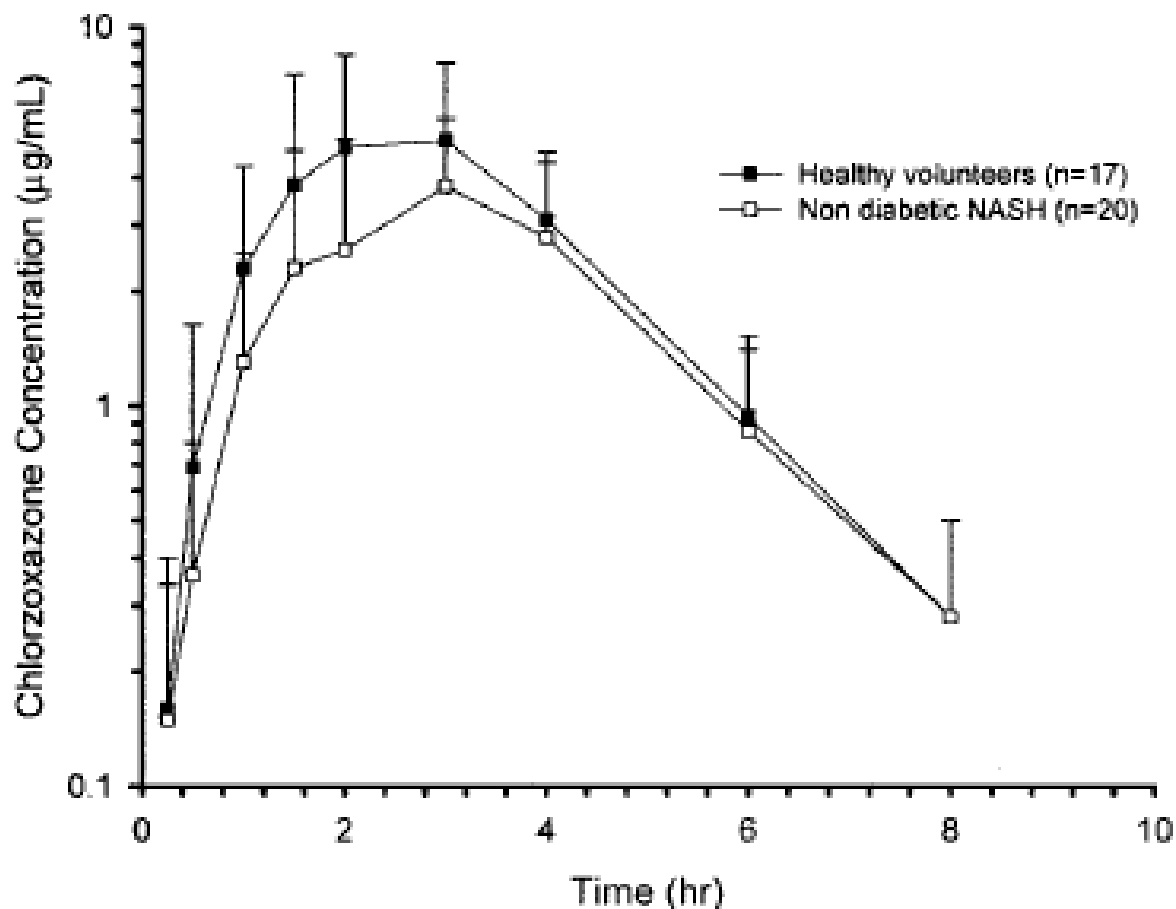


Fig. 1. Serum chlorzoxazone concentration (mean \pm SD) versus time plot for 20 nondiabetic subjects with NASH and 17 matched healthy volunteers following an oral dose of 500 mg of chlorzoxazone.

CYP2E1 in NASH patients

- Study of CYP2E1 before and after gastroplasty/weight loss, clearance of oral dose of chlorzoxazone, NASH confirmed by biopsy

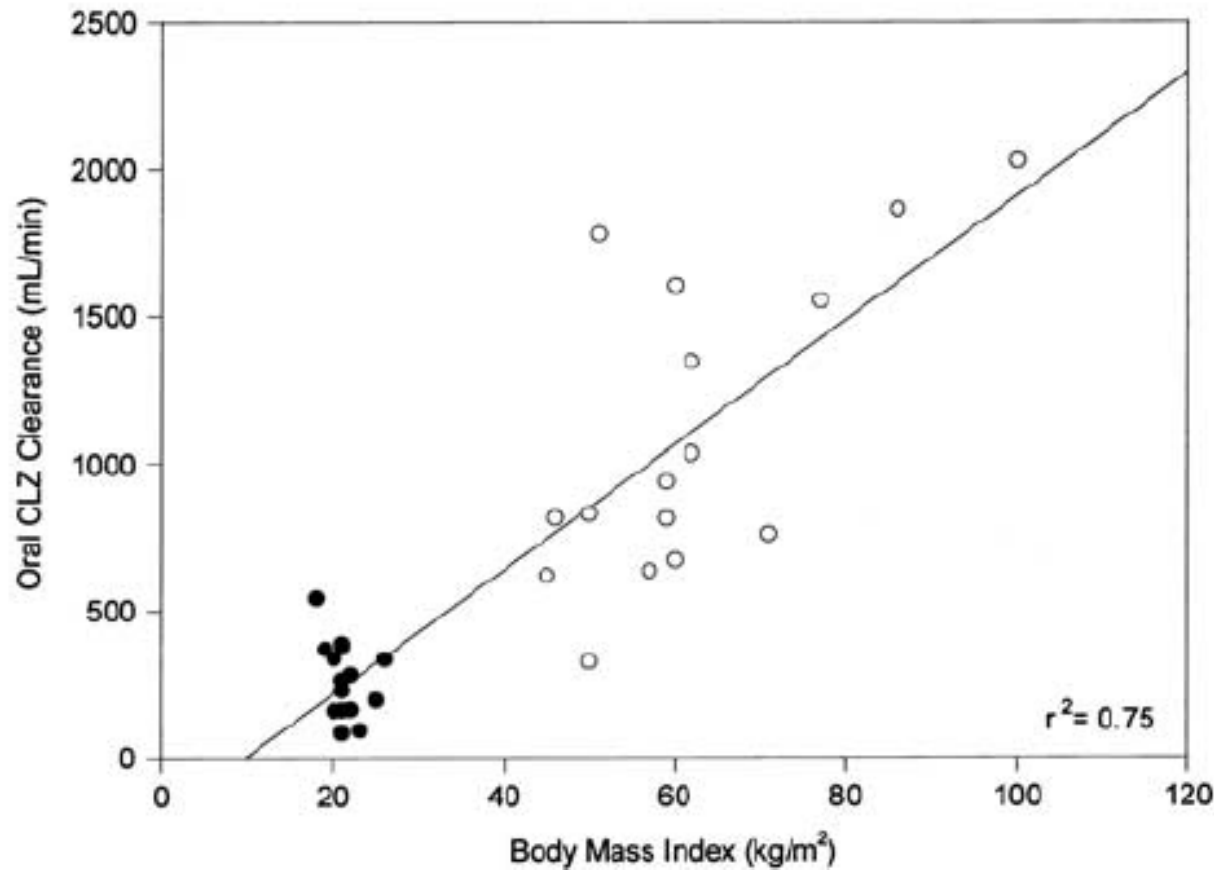
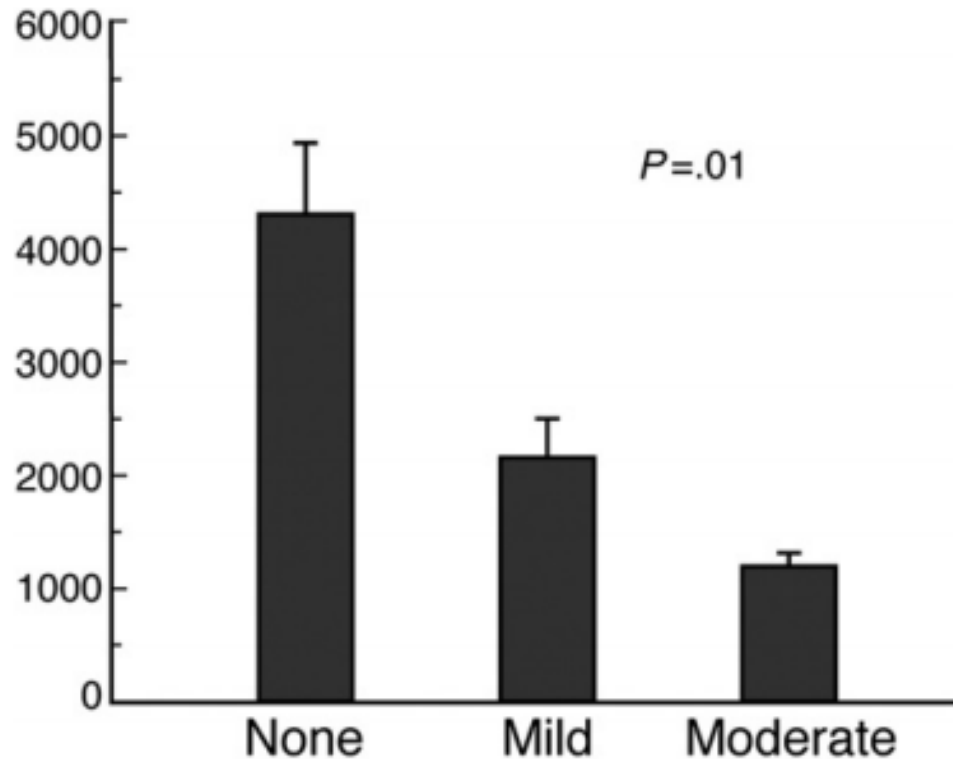


Fig. 1. Correlation of oral CLZ clearance with BMI. **Open boxes** represent obese subjects studied preoperatively. **Closed boxes** represent nonobese subjects.

CYP3A in patients with steatosis

Association between nonalcoholic hepatic steatosis and hepatic microsomal CYP3A enzyme activity (6 β -hydroxytestosterone formation) was studied in frozen livers. Steatosis was quantified from biopsies.



The reduction in microsomal activity CYP3A was not explained by the level of mRNA or immuno-quantification of the enzyme

CYP3A in patients with NASH

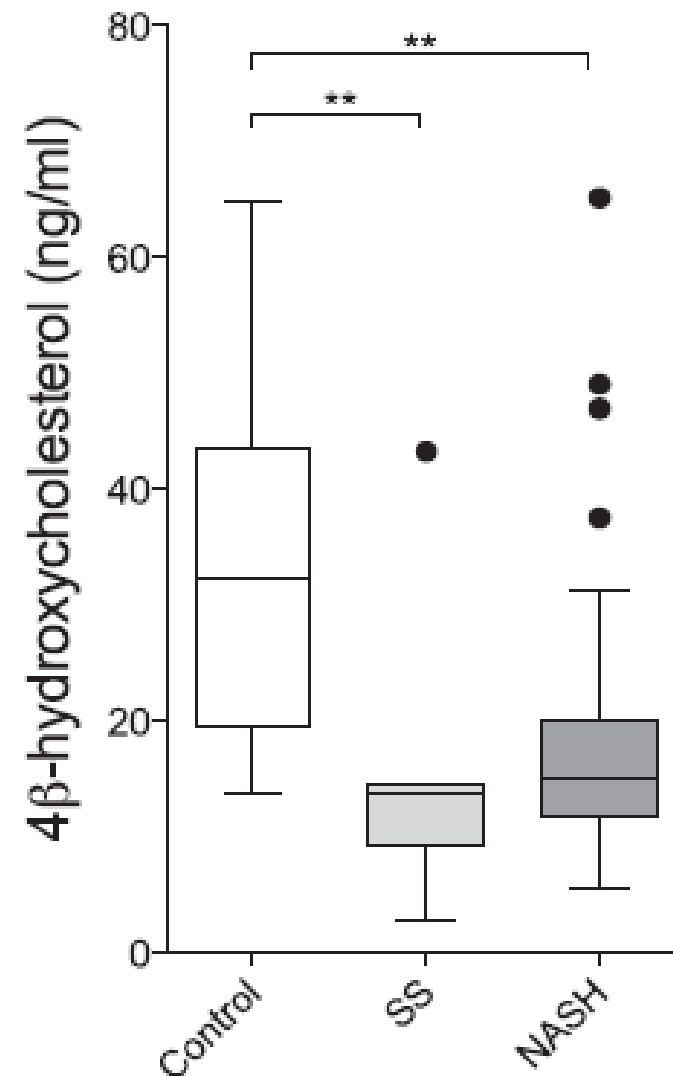
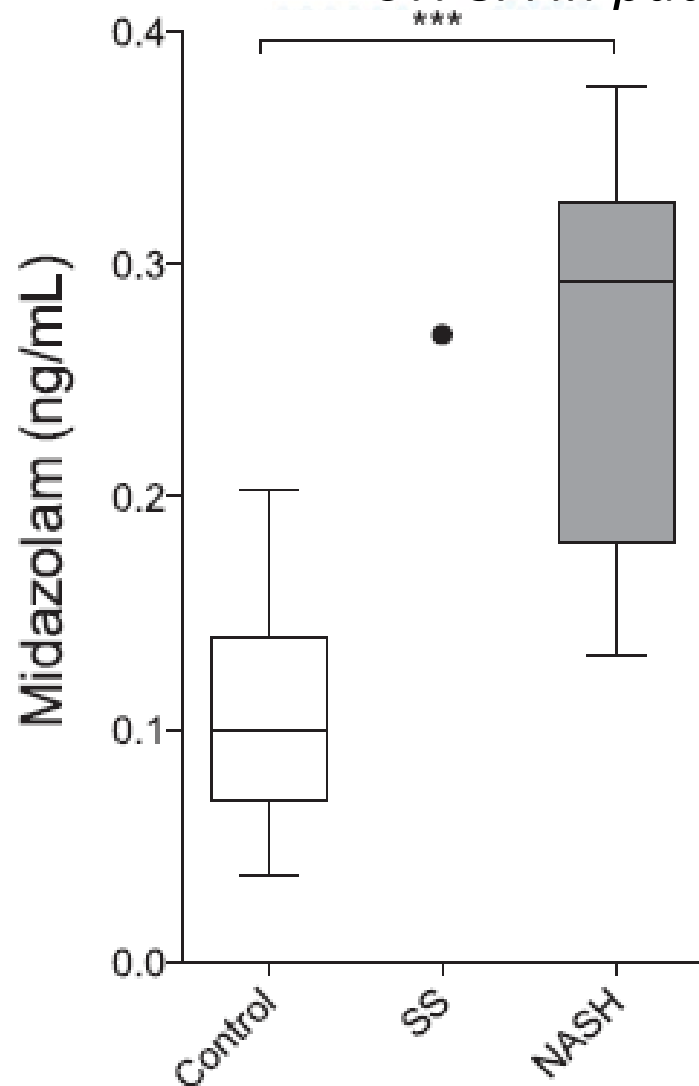
- In 1998 study by Weltman and co-workers, CYP2E1 and CYP3A4 immunostaining was conducted in liver sections from patients with NASH and compared with control liver sections. In regard to CYP2E1 authors findings were in agreement with studies we discussed earlier, namely CYP2E1 induction in NASH was demonstrated, but a reduction in CYP3A4 protein was also observed.

Weltman MD, Farrell GC, Hall P, et al. Hepatic cytochrome P450 2E1 is increased in patients with nonalcoholic steatohepatitis. *Hepatology* 1998;27:128–133.

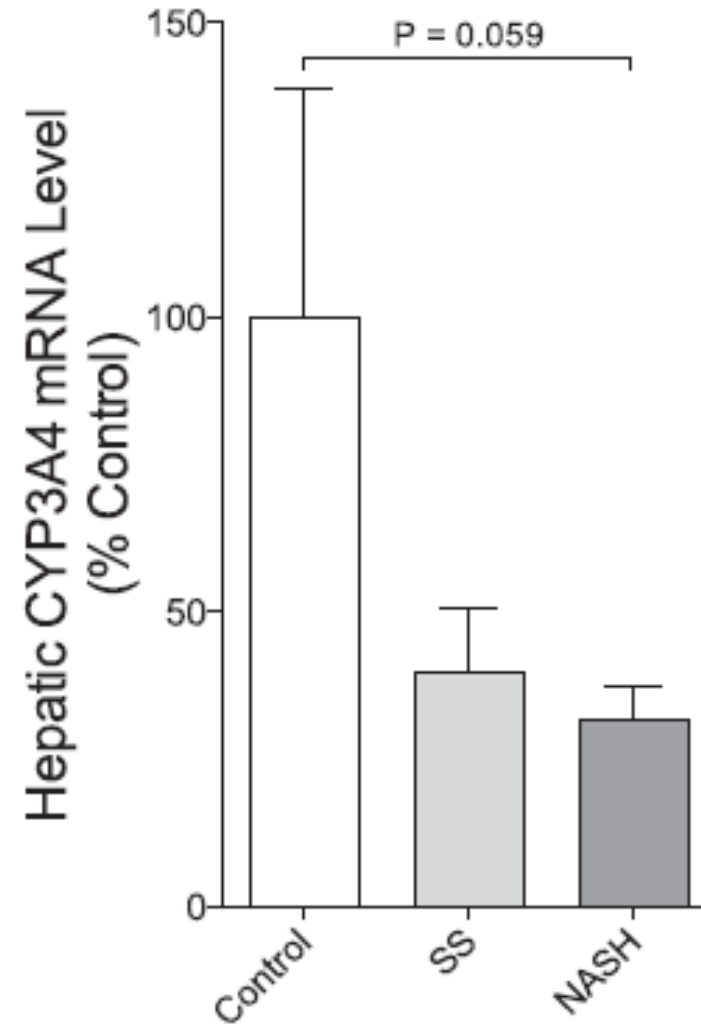
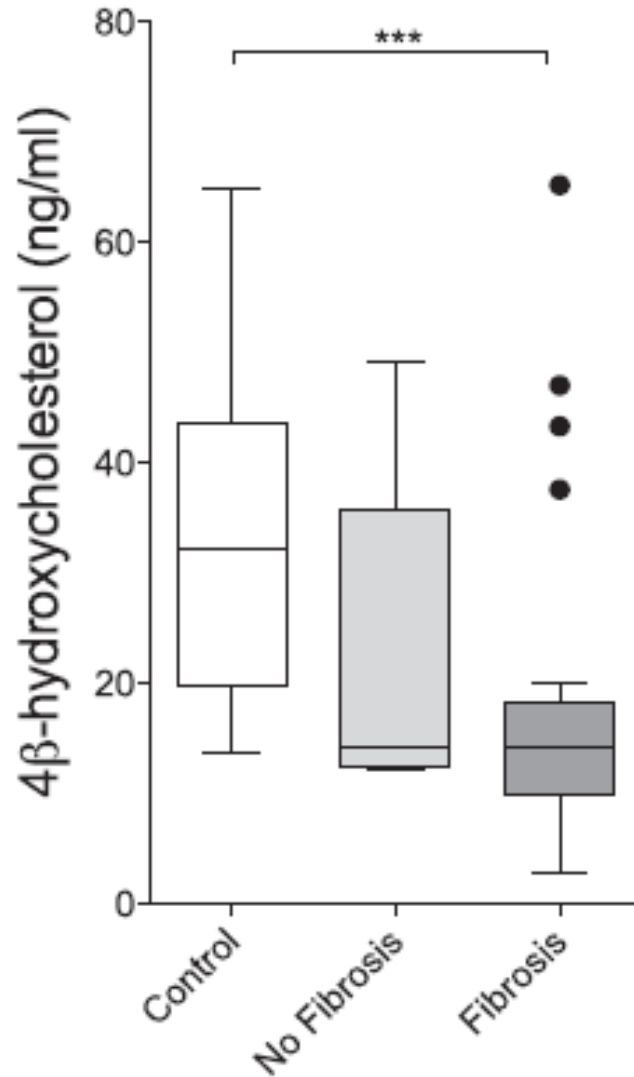
CYP3A in patients with NAFLD

- CYP3A Activity and Expression in Nonalcoholic Fatty Liver Disease.
- Study of human patients diagnosed based on biopsies, phenotyping with single oral dose of midazolam and 6 β -hydroxycholesterol an endogenous biomarker of CYP3A activity
- At the time of the study CYP3A4 reduction in patients with fibrosis and NAFLD with T2MD was known.
- Genotyping for CYP3A4*22, PPAR α , POR*28, PNPLA3 was conducted, mRNA or immuno-quantification of the proteins was not.
- NASH and fibrosis are associated with reduced expression and activity of CYP3A enzymes

CYP3A in patients with NAFLD



CYP3A in patients with NAFLD



CYP mRNA, protein, and enzymatic activity changes in NASH

CYP	mRNA	Protein	Activity	Ref
1A2	↔	↓	↓ (ex)	Fisher et al., 2009
2A6	↑ ↓	↑	↑	Fisher et al., 2009; Rubio et al., 2007
2C9	↔	↔	↑ (ex)	Fisher et al., 2009
2C19, 2D6	↔	↓	↓ (ex)	Fisher et al., 2009
2E1	↓ ↑	↓ ↑ ↑	↔ ↑ (ex) ↑ (in)	Fisher et al., 2009 Orellana et al., 2006 Weltman et al., 1998 Baker et al., 2010 Chalasani et al., 2003
3A	↔	↔ ↓	↔	Fisher et al., 2009 Weltman et al., 1998

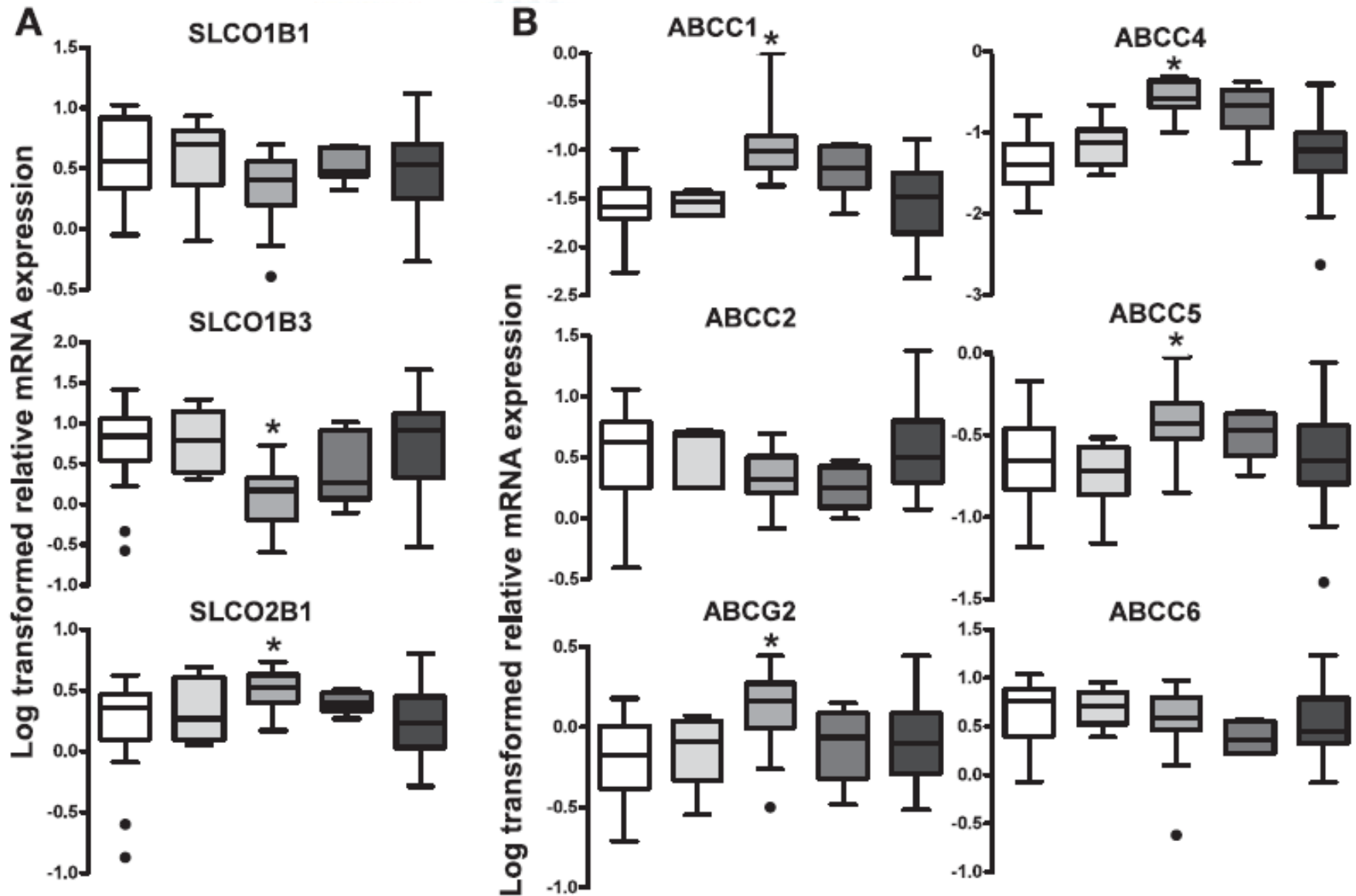
Membrane transporter changes in NASH

	mRNA	Protein	Localization
Uptake (Clarke, et al., 2014b)			
OATP1B1	↔	↑	-
OATP1B3	↓	↓	-
OATP2B1	↔	↔	-
Efflux (Hardwick et al., 2011)			
MRP1	↑	↑	-
MRP2	↔	↑	Internalized
MRP3	↔	↑	Membrane
MRP4	↑	↑	-
MRP5	↑	↑	-
MRP6	↔	↑	-
Pgp	↑	↑	Membrane
BCRP	↑	↑	Membrane

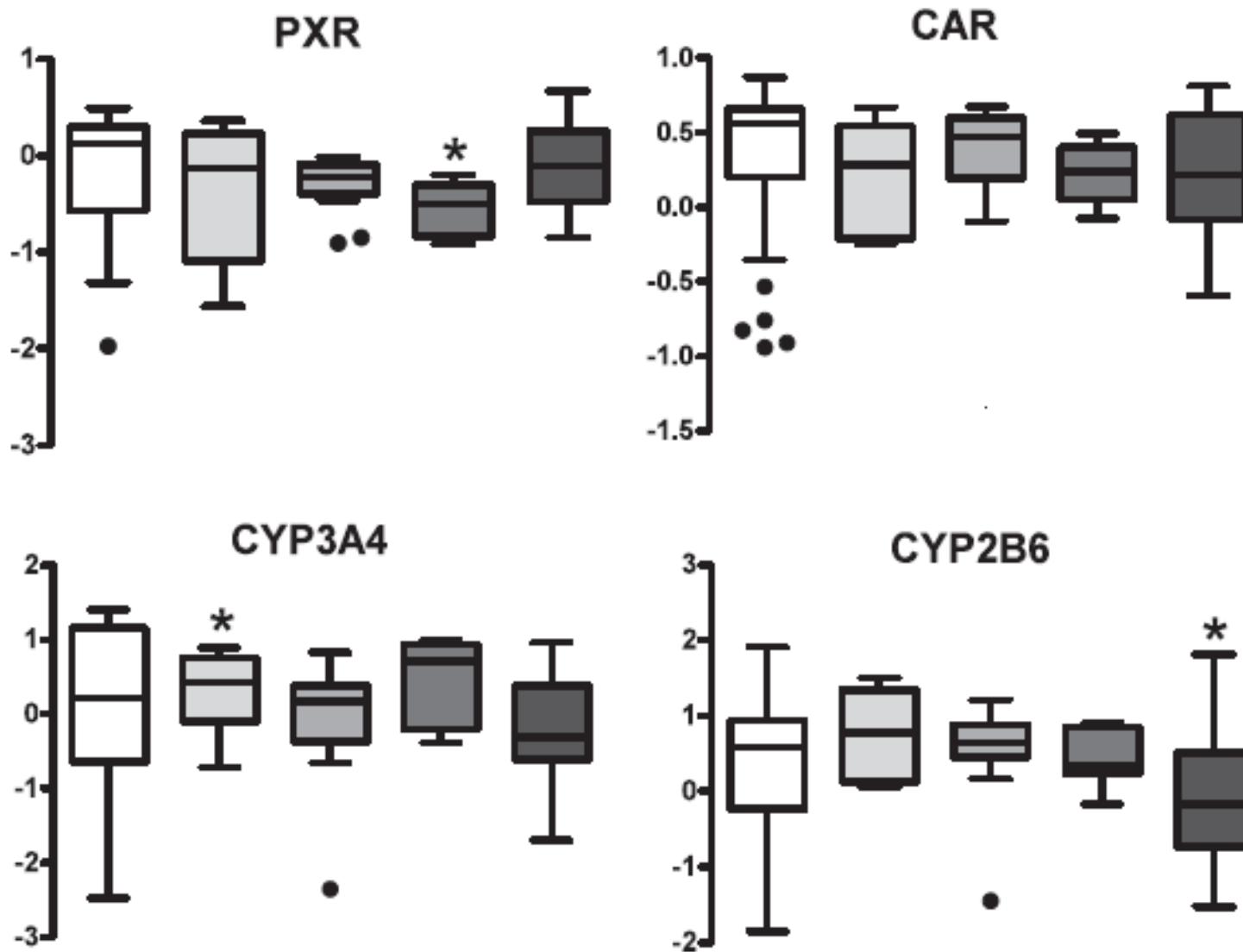
Alcohol cirrhosis alters nuclear receptor and drug transporters

- In this study about 80 samples of human liver were evaluated by QuantiGene Plex (mRNA) and Western blotting
- Samples were categorized as normal, steatosis, alcohol cirrhosis, diabetic cirrhosis and diabetes. NASH as a specific category or identification was not made.

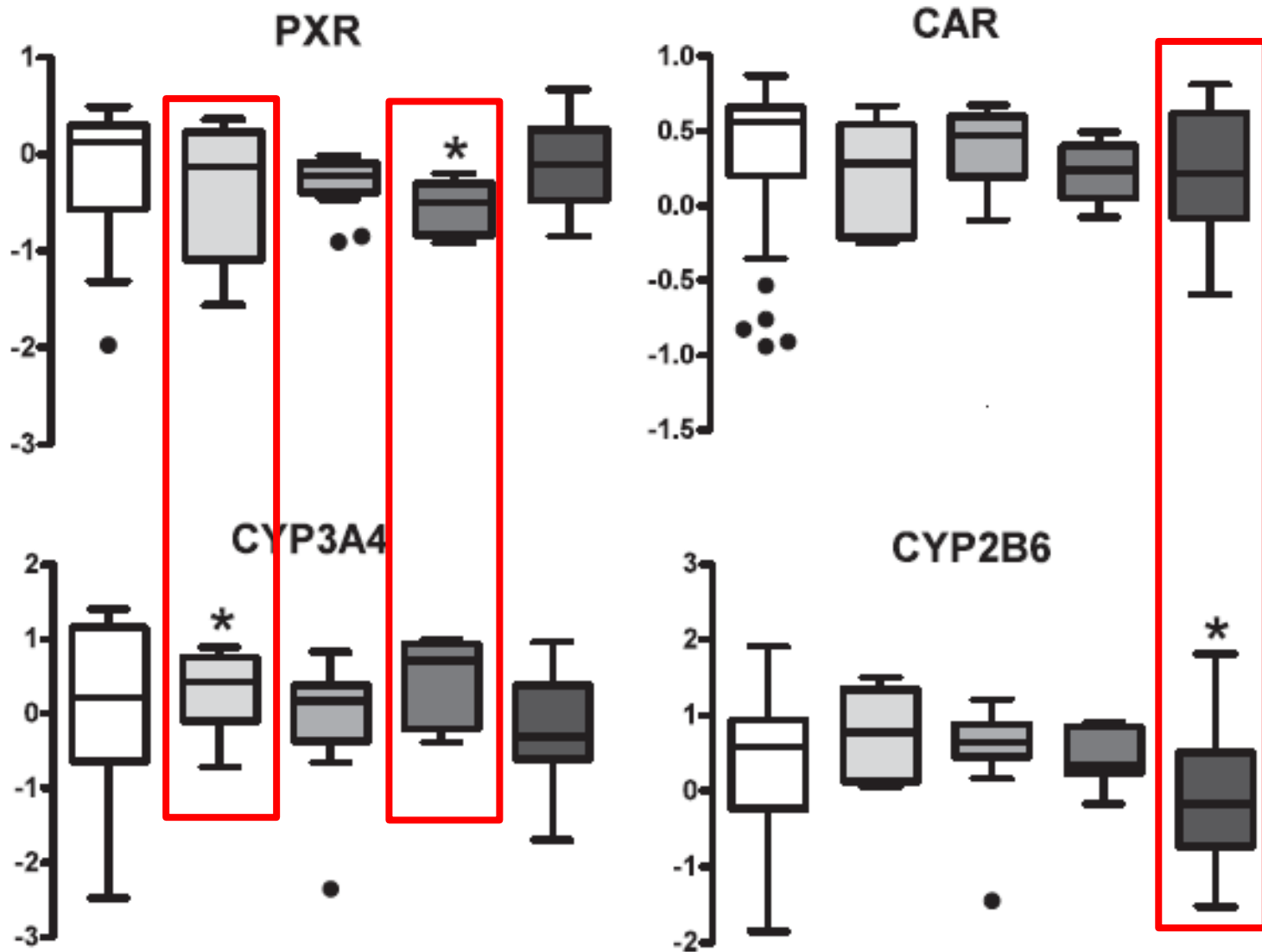
Alcohol cirrhosis alters nuclear receptor and drug transporters



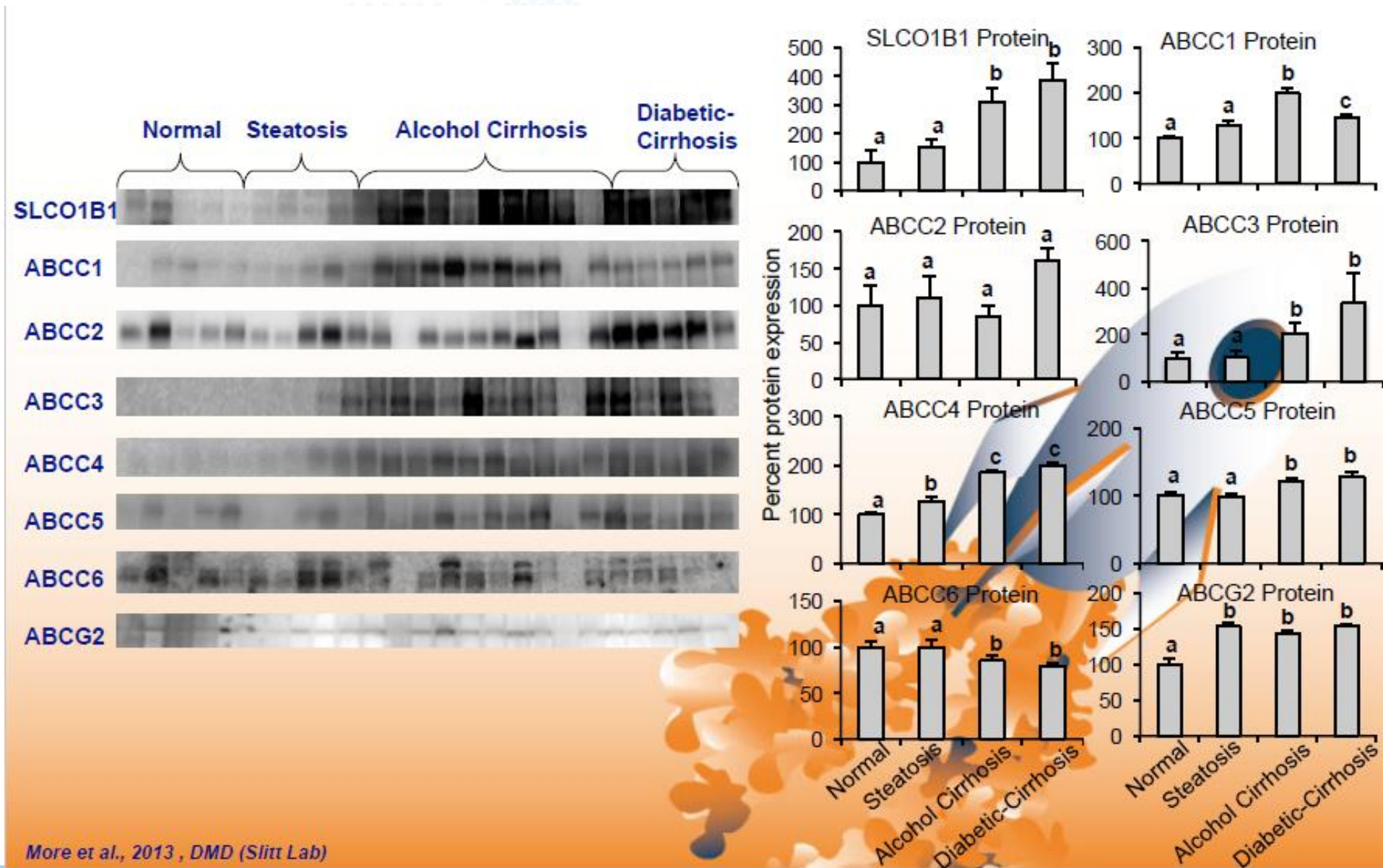
Alcohol cirrhosis alters nuclear receptor and drug transporters



Alcohol cirrhosis alters nuclear receptor and drug transporters



Alcohol cirrhosis alters nuclear receptor and drug transporters



MRP2 and MRP3 in pediatric NASH patients

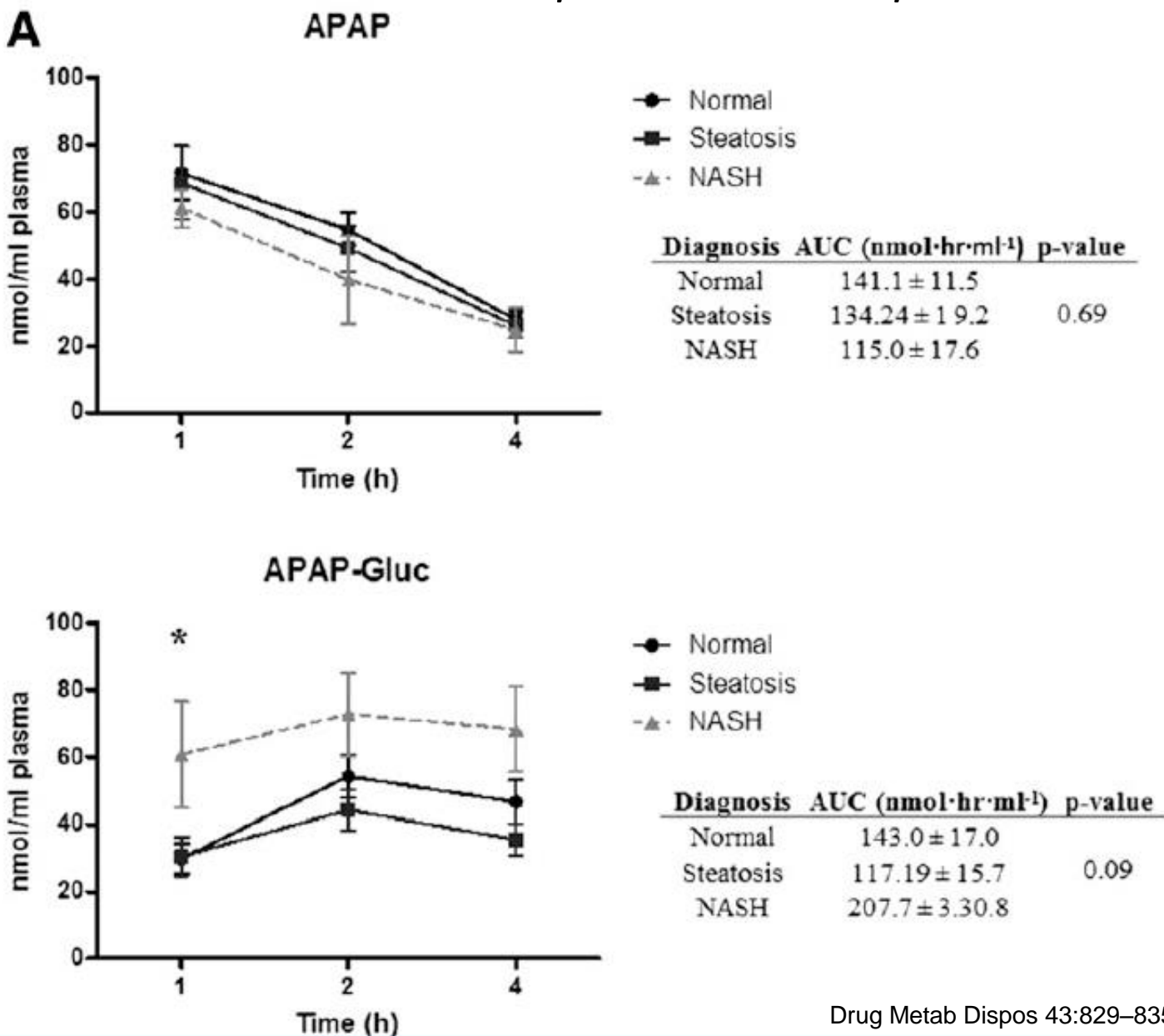
Preliminary pilot study - children with NASH tend to have increased retention of the metabolite APAP-gluc in systemic circulation, along with increased excretion into the urine.

A dysregulation of the hepatic membrane transporters MRP2 and MRP3 is a potential mechanism for these observations.

Immunoblotting for MRP3 (basolateral, in adult livers) and immunocytochemistry for MRP2 (canalicular) was provided. MRP3 was increased, MRP2 displayed “altered localization”.

Liver biopsies were conducted

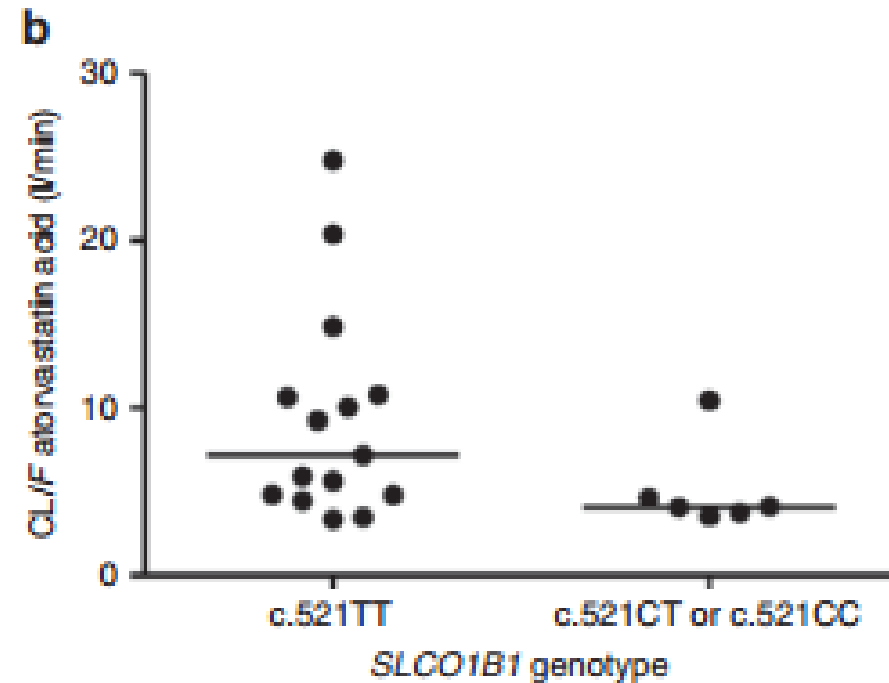
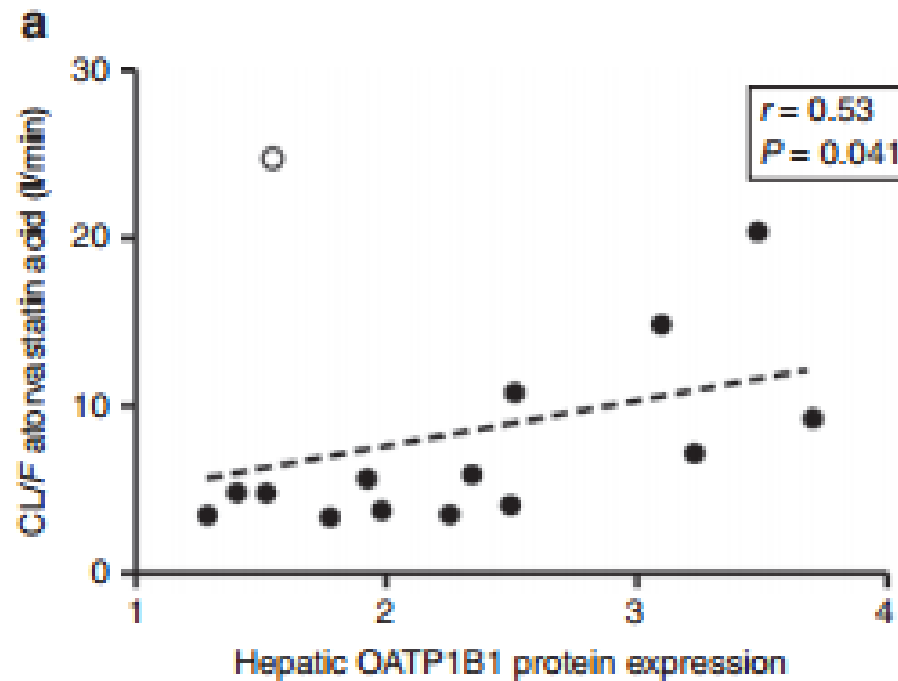
MRP2 and MRP3 in pediatric NASH patients



Pharmacokinetics of atorvastatin in obese subjects

- Pharmacokinetics of atorvastatin;
- OATP1B1, MRD1 and CYP3A4 determine disposition of this statin;
- Paired liver and small-intestinal biopsies;
- BMI 45 (34-59), T2DM, hypertension, hypercholesterolemia, sleep apnea, hypothyroidism, but no data on alcohol;
- OATP1B1 genotyped for reduced activity allele c.521T→C;
- Study did not have healthy control group;
- Main conclusion - OATP1B1 genotype was a major determinant of atorvastatin clearance;

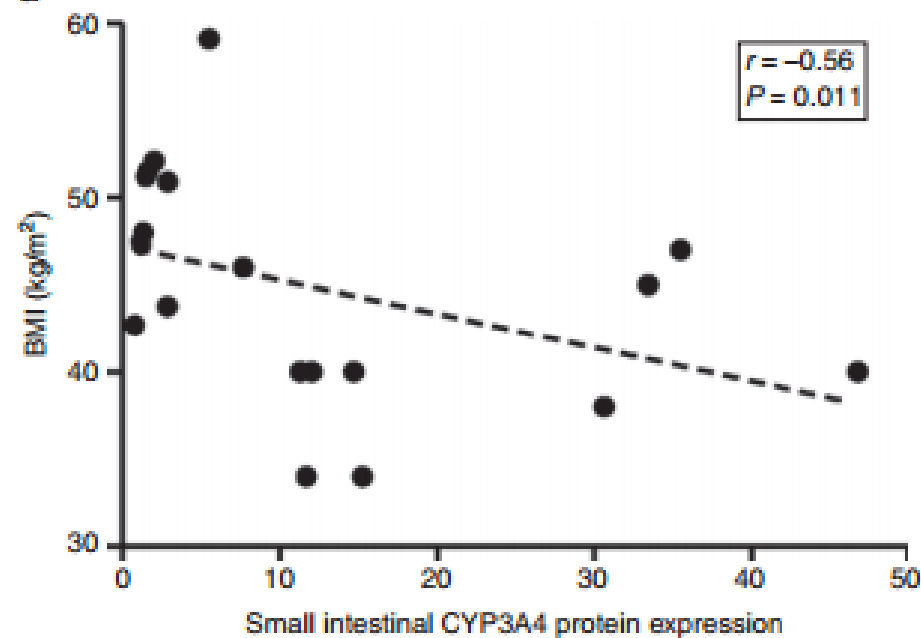
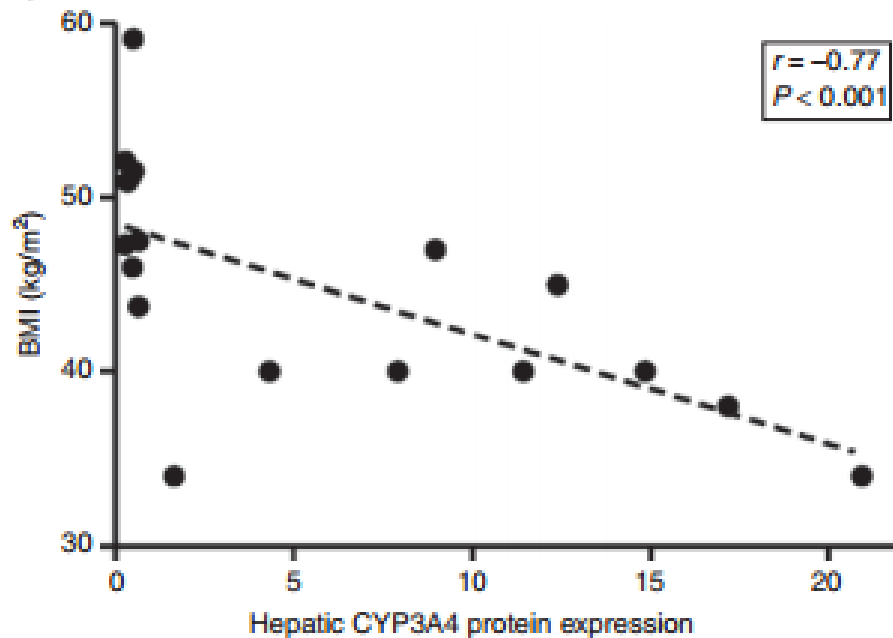
Pharmacokinetics of atorvastatin in obese subjects



OATP1B1 genotype was a major determinant of atorvastatin clearance

Pharmacokinetics of atorvastatin in obese subjects

- BMI was negatively correlated with hepatic CYP3A4 protein, as expected in NAFLD or NASH, but also with the intestinal enzyme;
- BMI was not associated with levels of expression of OATP1B1 or MDR1 in the liver or small intestine.

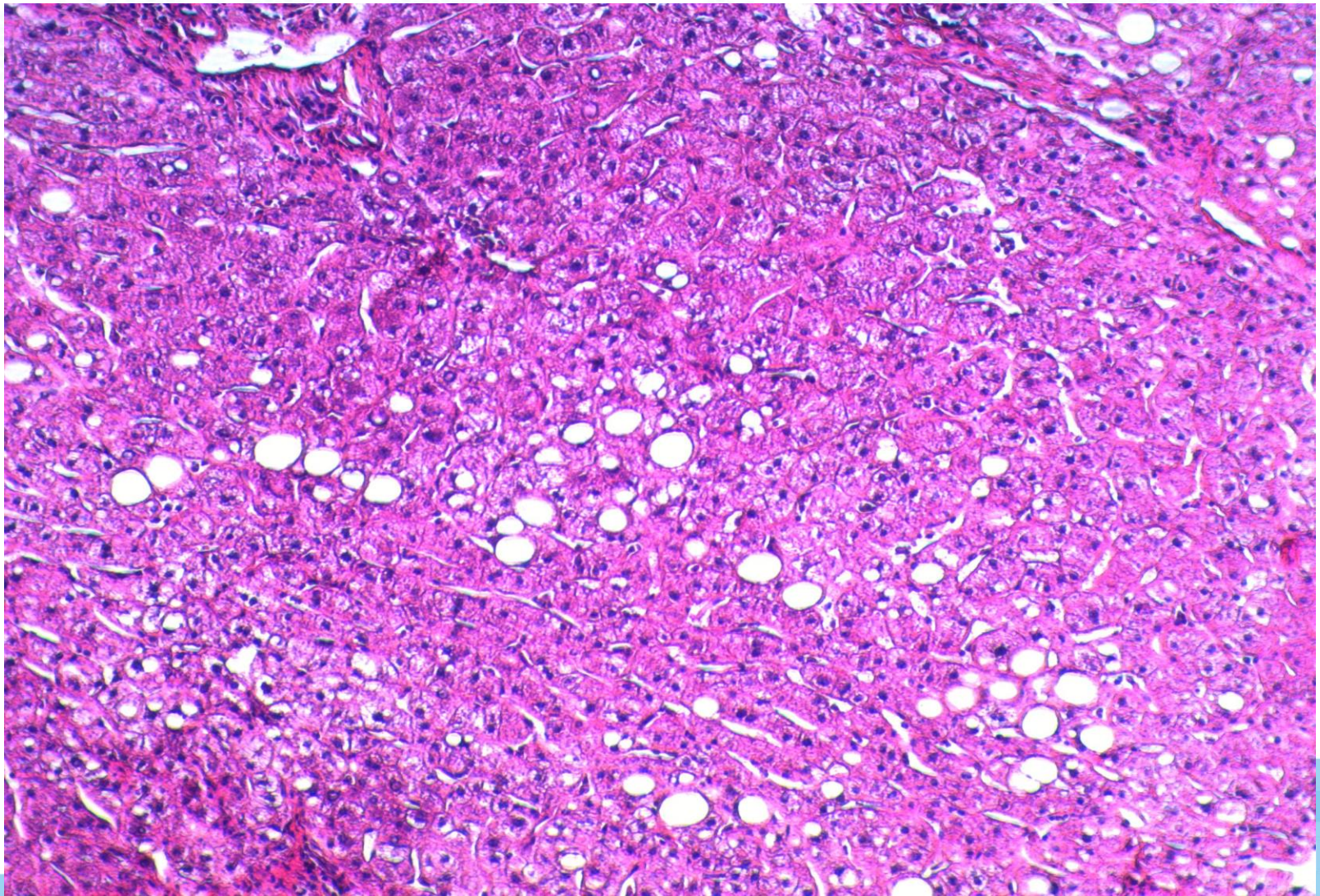


Introduction to Research Biobank

- XenoTech processes non-transplantable human livers into isolated hepatocytes, Kupffer cells and subcellular fractions – S9, microsomes, cytosol, lysosomes, etc.
- Support of basic research into liver diseases is part of our mission. Tissues, we accumulated over the years, may be of value for advancement of human health. This motivated us to establish the Research Biobank.
- Research Biobank collects, characterizes, stores and distributes human liver tissues.

Non-alcoholic steatohepatitis (NASH)

Donor H1069, diagnostic features are macrovesicular fat >5%, lobular inflammation, fibrosis and ballooning necrosis



Nonspecific histopathological changes sometimes seen in liver biopsy

1. Mild and focal inflammatory infiltrate in the portal tracts, mild portal fibrosis
2. Focal (rare) liver cell necrosis or occasional acidophilic bodies
3. "Surgical hepatitis"
4. Prominent Kupffer cells
5. Kupffer cell pseudogranuloma
6. Extramedullary hematopoiesis
7. Circulating megakaryocytes
8. Microgranulomas or occasional noncaseating epithelioid granulomas
9. Focal, mild macrovesicular steatosis
10. Sinusoidal dilatation (focal and without zonal distribution)
11. Mild bile duct epithelial change
12. Lipofuscin pigment
13. Hemosiderin in Kupffer and sinusoidal endothelial cells

“Surgical hepatitis”

Biopsy obtained at the end of an abdominal surgical procedure, either as a needle biopsy or, more often, as a wedge, can have clusters of polymorphonuclear leukocytes irregularly distributed in sinusoids. These clusters, so-called surgical hepatitis, most often seen in zone 3, near the terminal hepatic venule (central vein), or immediately beneath the liver capsule. It is thought that surgical hepatitis is caused by foci of anoxia within the liver or possibly by mechanical injury, perhaps from surgical retractors. Occasionally true liver cell necrosis with rare acidophilic body formation can also be seen, but there is no true hepatitis.

*Characterization provided on data sheets (1)***HHPL.NT Lot No. H1305**

Research Biobank Human Liver Pre-Lysate

Diagnosis: Normal

0.5 g of tissue in 2.0 mL

Suspension Medium: Suitable buffer of customer's choosing

Donor Information

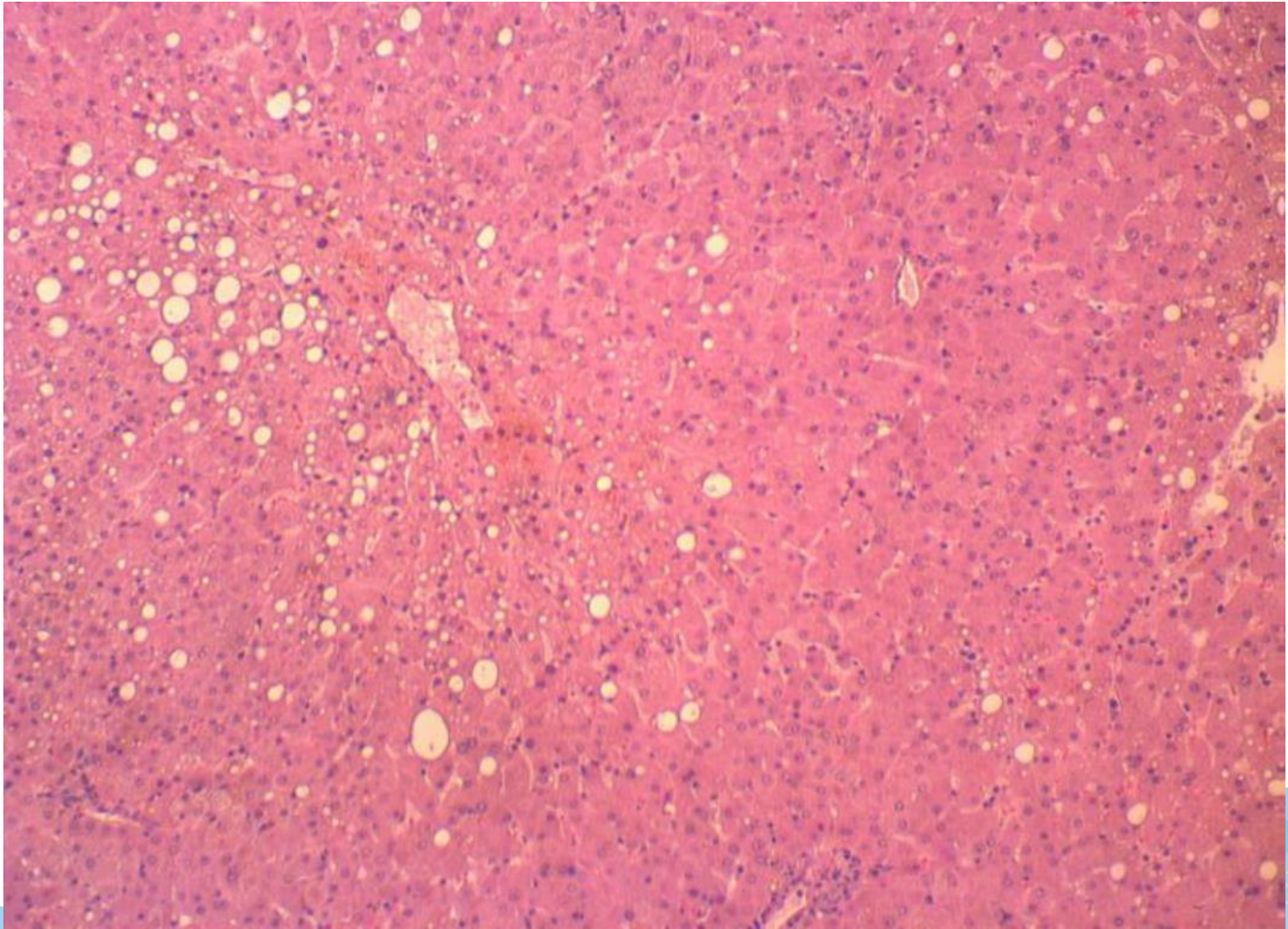
Macro Fat %	Age	Gender	Race	BMI	Alcohol Use	Alcohol Frequency	Diabetes	Hepatocytes Isolated
1	38	Male	Caucasian	25.3	Yes	Heavy	No	Yes

Serology Information

Antibody to Cytomegalovirus (CMV):	Positive
Human Immunodeficiency Virus (HIV):	Negative
Hepatitis B Surface Antigen (HbsAg):	Negative
Antibody to Hepatitis C Virus (HCV):	Negative

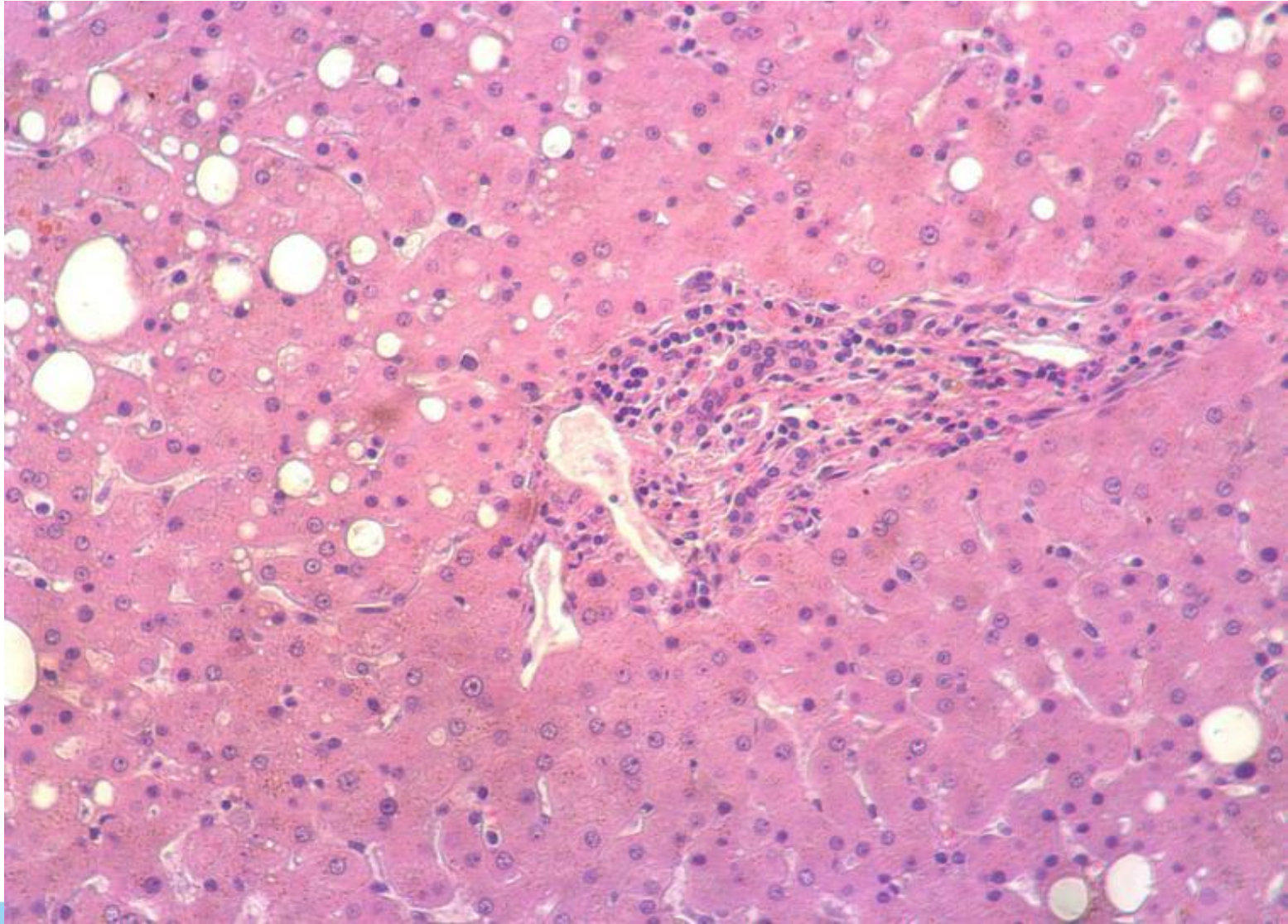
Characterization provided on data sheets (2)

H1305, normal, lower magnification, H&E stain



Characterization provided on data sheets (3)

H1305, normal, higher magnification, H&E stain



Current holdings of the Biobank

- Adult
 - normal 53
 - steatosis 45
 - without history of alcohol use
 - steatohepatitis 14
 - NASH 7
 - diabetes 26
- Pediatric 13 (up to 4 years of age)
- Inventory is expanding