Imaging Studies with the Transporter Probe ^{99m}Tc-Mebrofenin Reveal Altered Hepatic Exposure in Patients with Non-Alcoholic Steatohepatitis (NASH)

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Conflict of Interest Disclosure

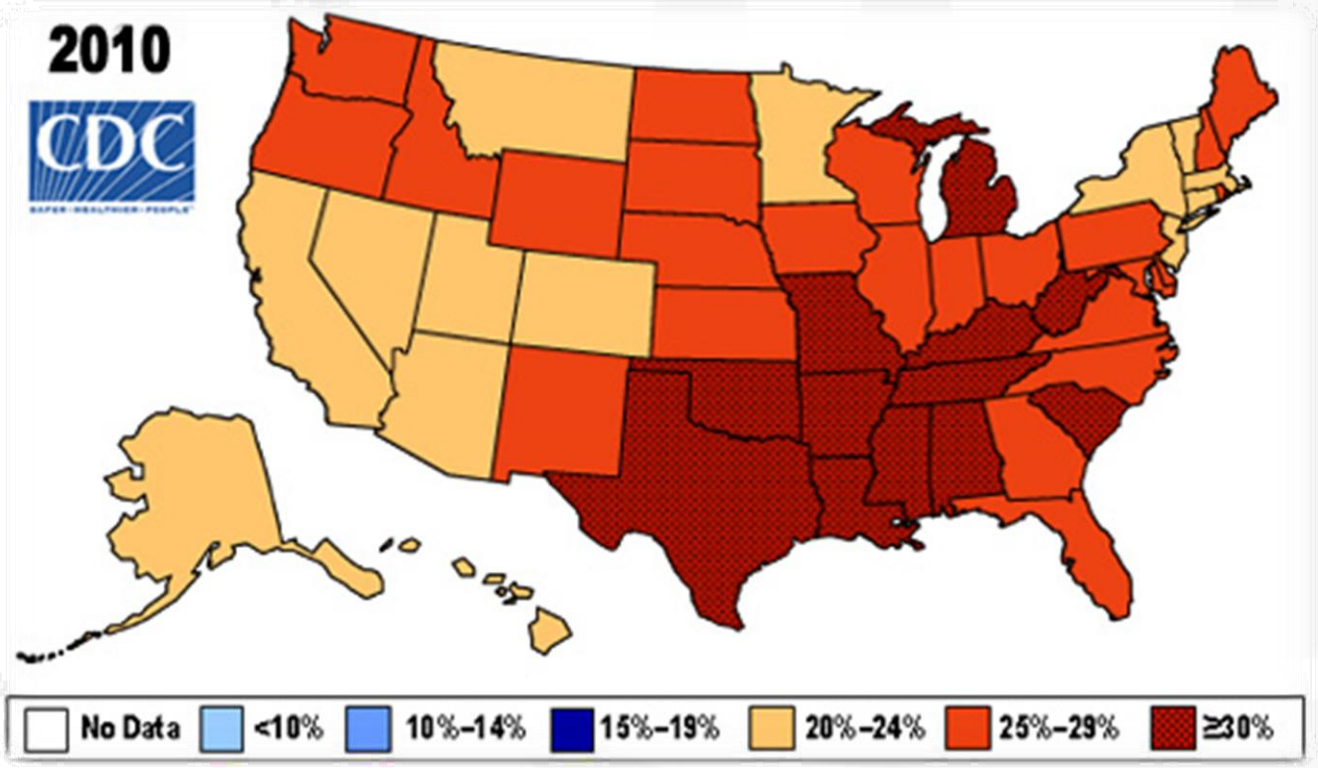
- The Brouwer lab receives research funding from the National Institutes of Health, National Institute of General Medical Sciences [Grant R01 GM041935-24], Intercept Pharmaceuticals, and Otsuka Product Development & Commercialization
- Dr. Kim Brouwer is co-inventor of the sandwich-cultured hepatocyte technology for quantification of biliary excretion (B-CLEAR®) and related technologies, which have been licensed exclusively to Qualyst Transporter Solutions, LLC

Outline

- Background
  - The Obesity Epidemic
    - Non-Alcoholic Fatty Liver Disease (NAFLD)
    - Non-Alcoholic Steatohepatitis (NASH)
  - NASH-mediated Alterations in Hepatic Transporters
  - ^{99m}Tc-Mebrofenin
    - Clinical Probe to Assess Hepatic Transporter Function
- Results
  - Imaging Hepatic Exposure of ^{99m}Tc-Mebrofenin in Patients with Biopsy-confirmed NASH
- Conclusions

The Obesity Epidemic

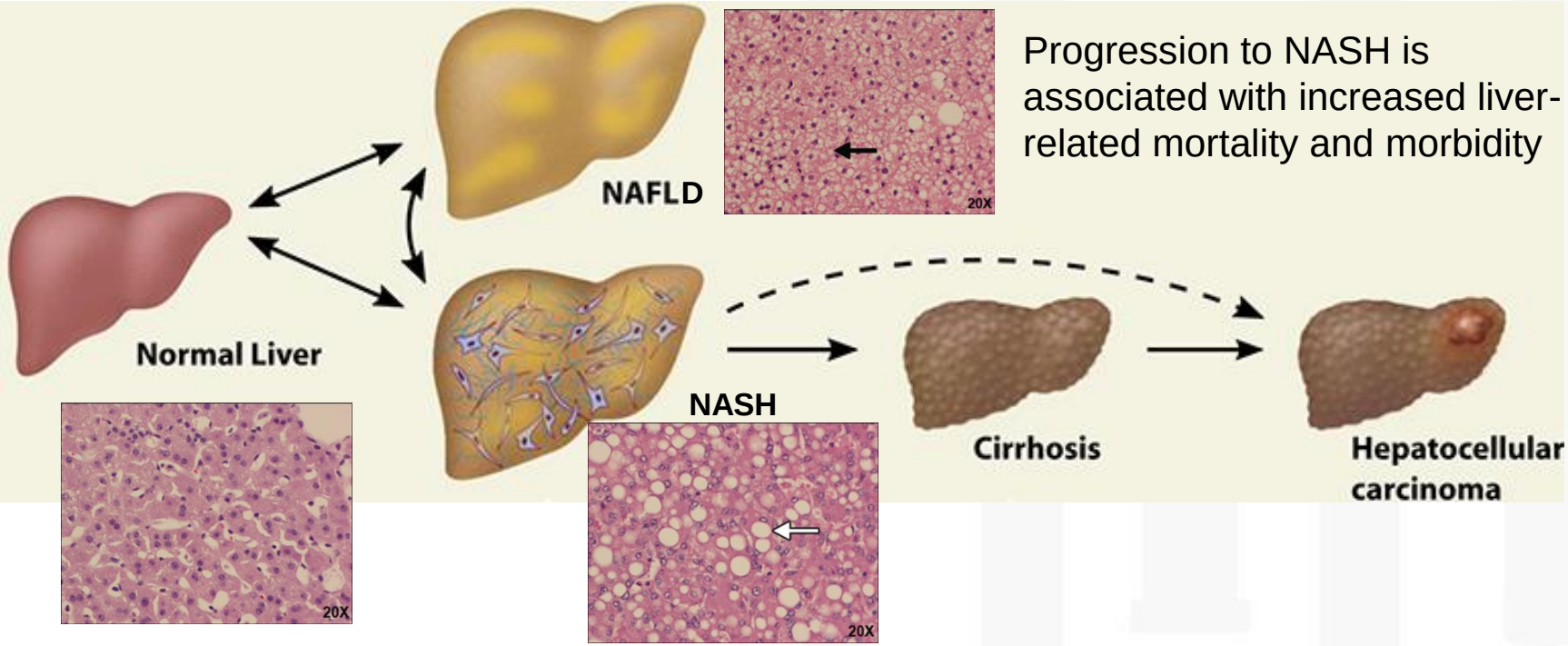
- Associated with metabolic syndrome
  - Includes: dyslipidemia, hypertension, type II diabetes, and obesity
  - 90% of NAFLD patients have at least one component



Nakahara et al., Hepatol Res **42**:1065, 2012;
Tikkanen et al., Int J Cardiol **168**:3846, 2013;
Sanyal et al., N Engl J Med **362**:1675, 2010;
Torres et al., Hepatol **54**:1631, 2011

The Spectrum of NAFLD

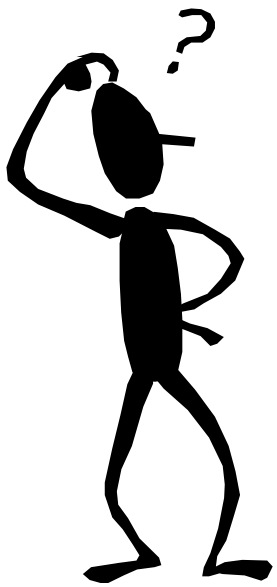
- Steatosis and steatohepatitis comprise Non-Alcoholic Fatty Liver Disease (NAFLD) and Non-Alcoholic Steatohepatitis (NASH)
- In the US, the prevalence of NAFLD is ~30%; NASH prevalence is ~3-5%



Liver histopathology reveals progression from fatty liver to steatosis, hepatocyte ballooning and lobular inflammation

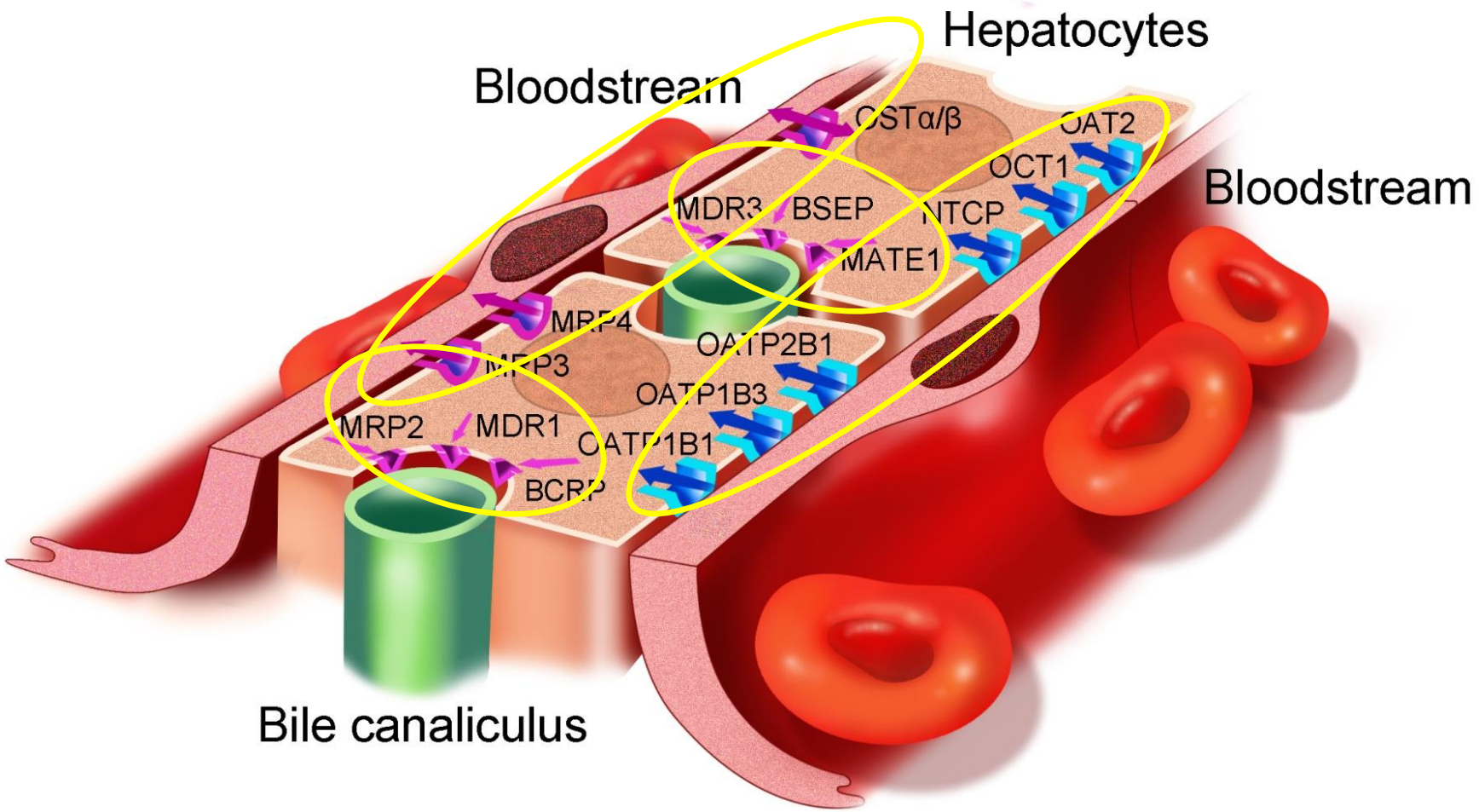
Vernon et al., Aliment Pharmacol Ther 34:274, 2011; Hardwick R et al., Drug Metab Dispos, **39**:2395, 2011; Williams CD et al., Gastroenterology, **140**:124, 2011

What is the Impact of NASH on:



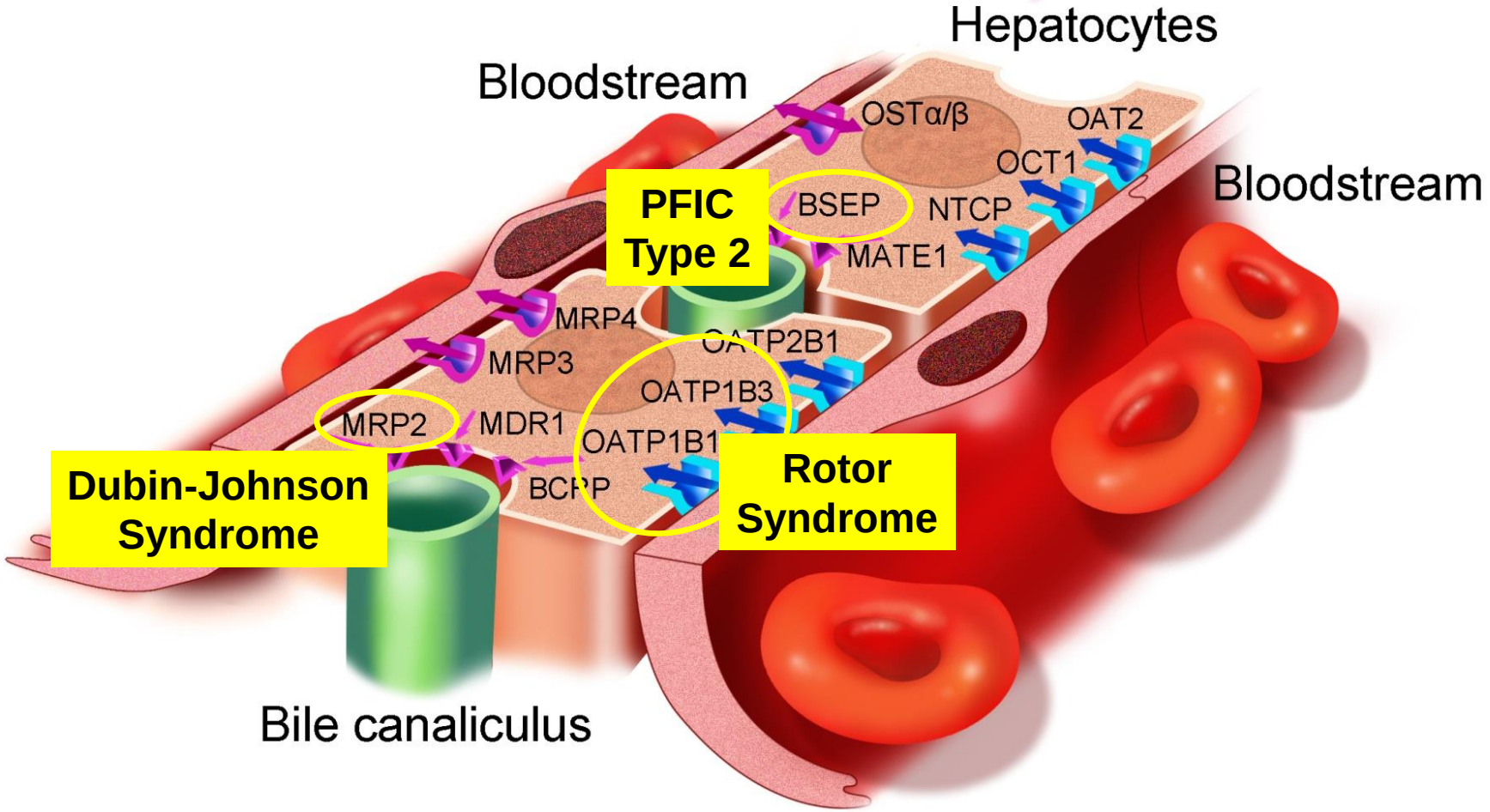
- Hepatic transport protein expression?
- Hepatic transporter function?
- Hepatic exposure to drugs and metabolites?

Hepatic Uptake and Efflux Transporters



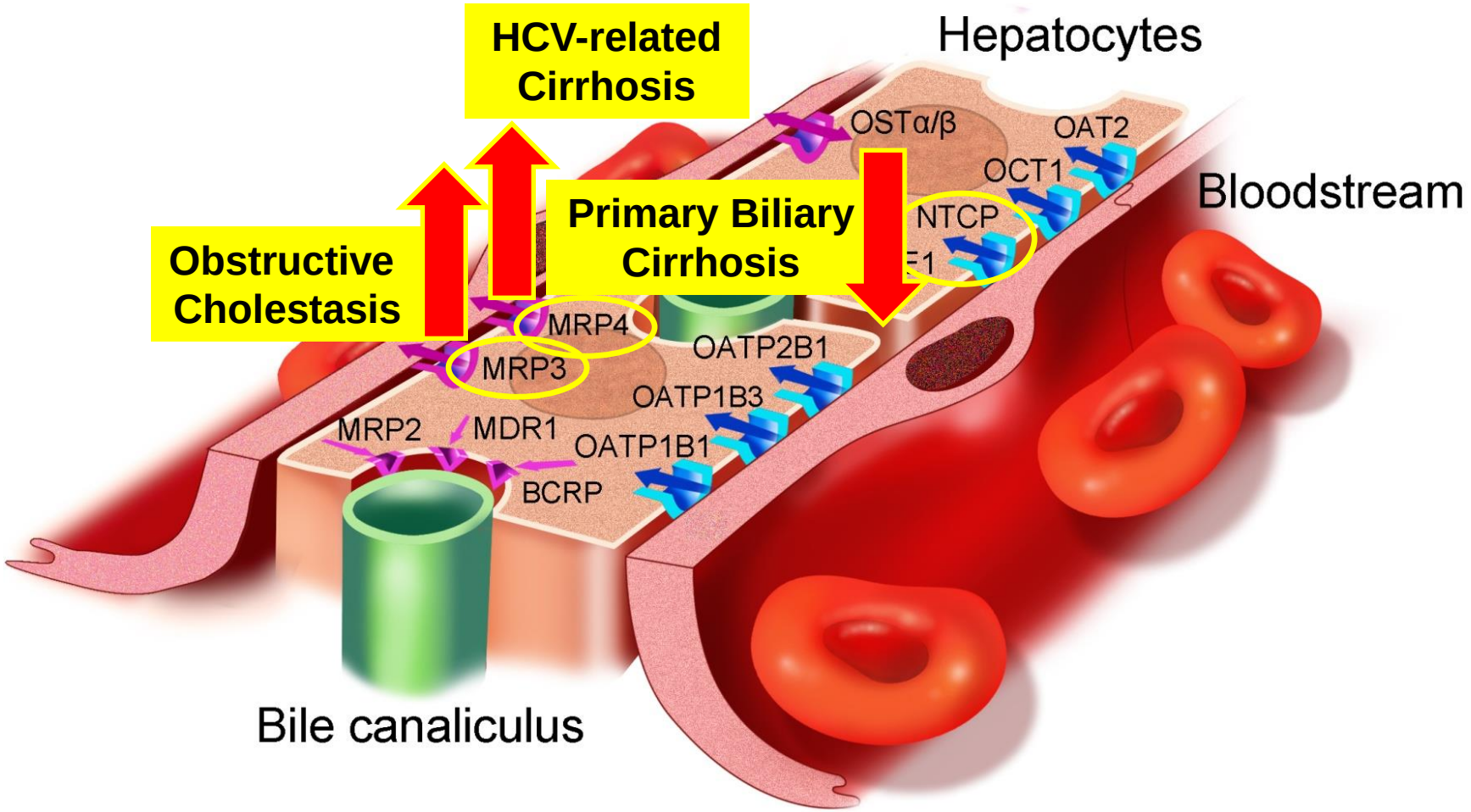
Köck and Brouwer, Clin Pharmacol Ther, **92**:599, 2012
(Adapted from Ho and Kim, Clin Pharmacol Ther, **78**:260, 2005)

Hepatobiliary Transport Proteins as Underlying Factors in Hepatic Disease



Köck and Brouwer, Clin Pharmacol Ther, **92**:599, 2012
van de Steeg et al., J Clin Inv, **122**:519, 2012

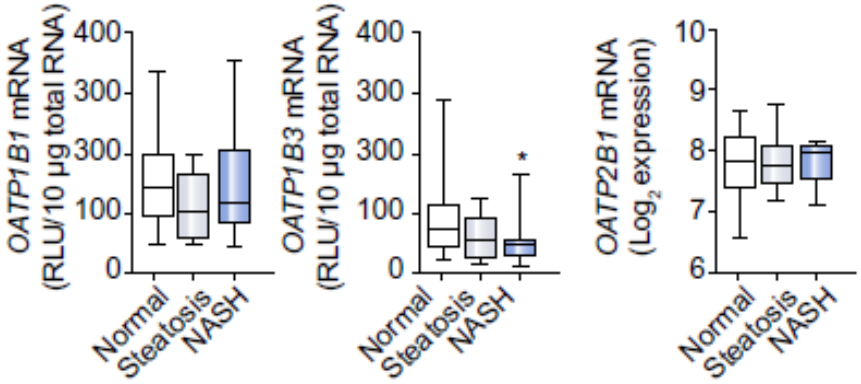
Hepatic Disease-Associated Alterations in Hepatobiliary Transport Proteins



Chai et al., Hepatology **55**:1485, 2012; Ogasawara et al., Drug Metab Pharmacokinet, **25**:190, 2010
Zollner et al., Liver Intl, 2007; Takeyama and Sakisaka, Hepatology Res, **42**:120, 2012

Altered Expression of Hepatic OATPs in NASH

mRNA



Clarke et al., J Hepatol, **61**:139, 2014

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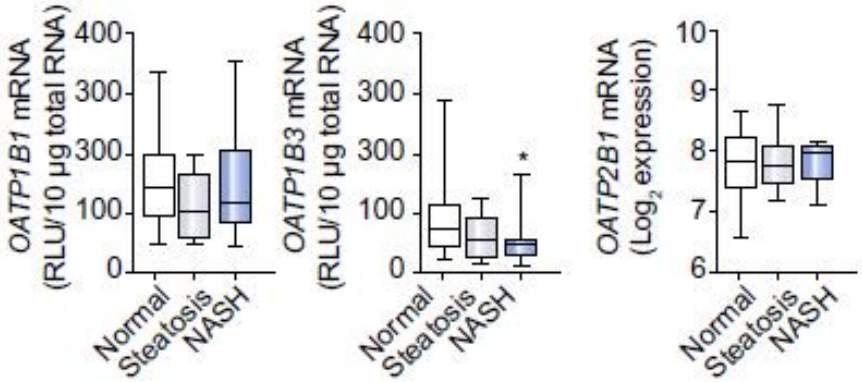
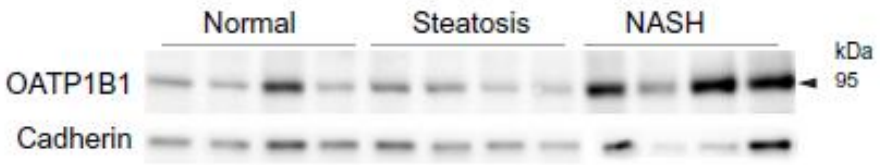
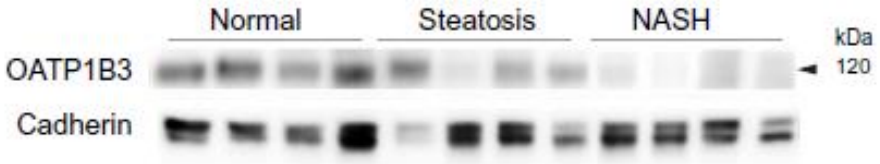
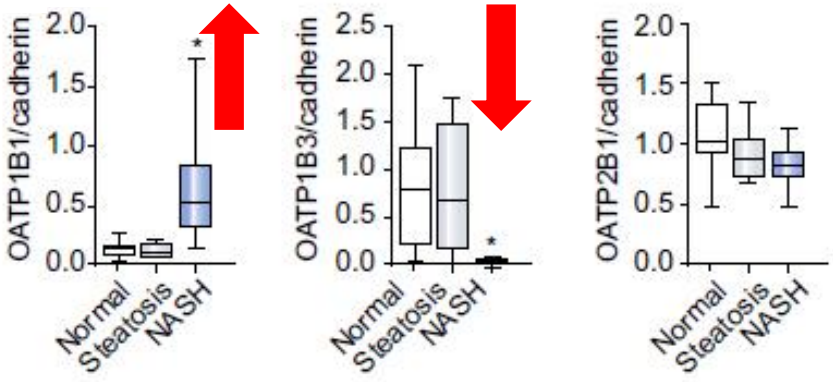
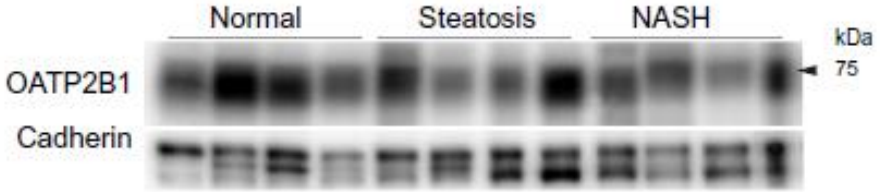






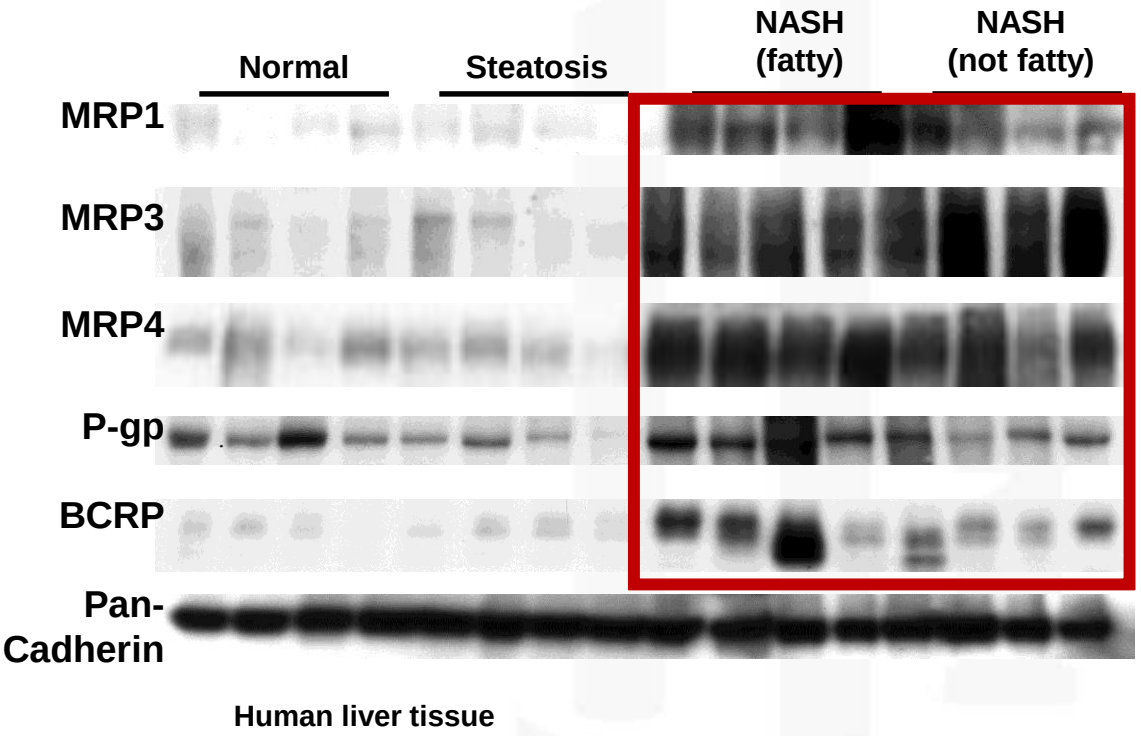
Protein





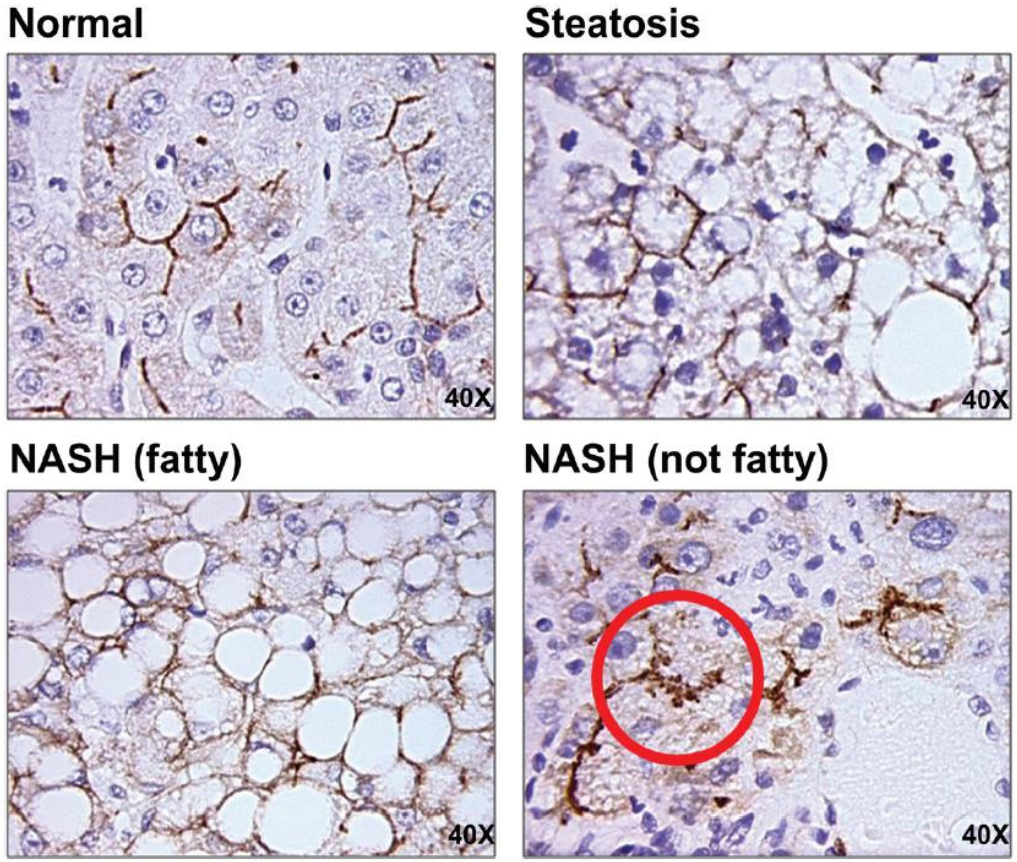
Clarke et al., J Hepatol, **61**:139, 2014

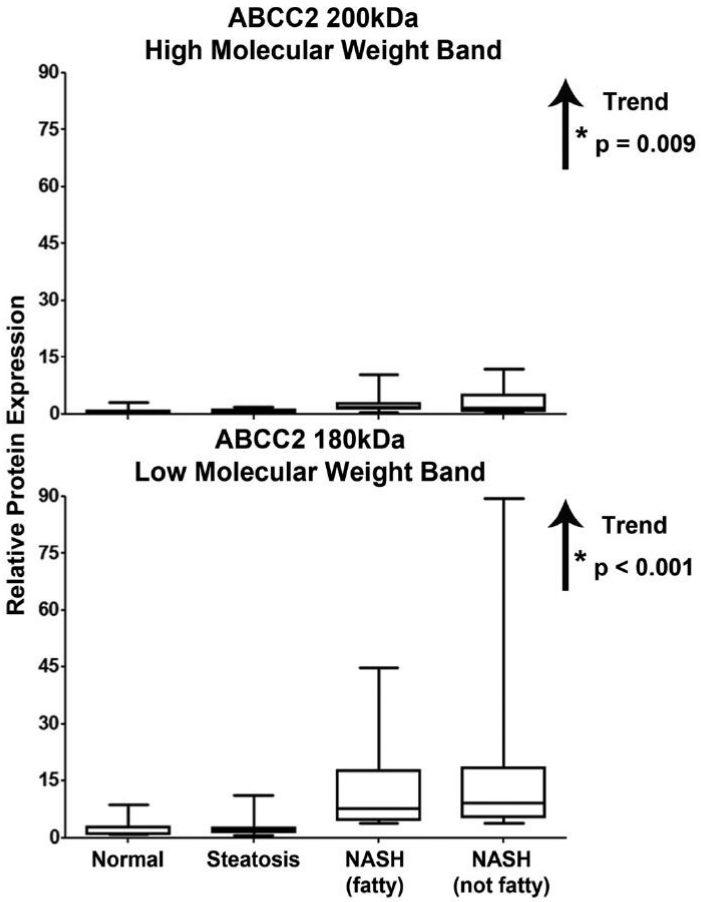
Increased Expression of Hepatic Efflux Transporters in NASH

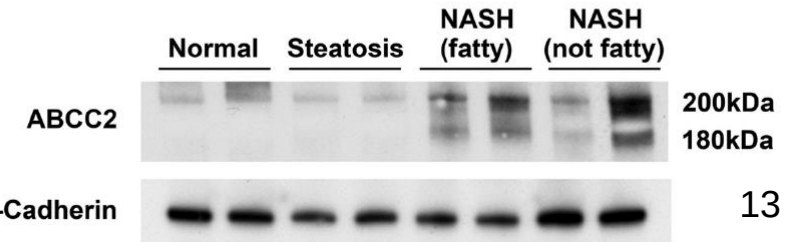


Hardwick et al., Drug Metab Dispos, **39**:2395, 2011

Altered MRP2 Localization and Expression in NASH

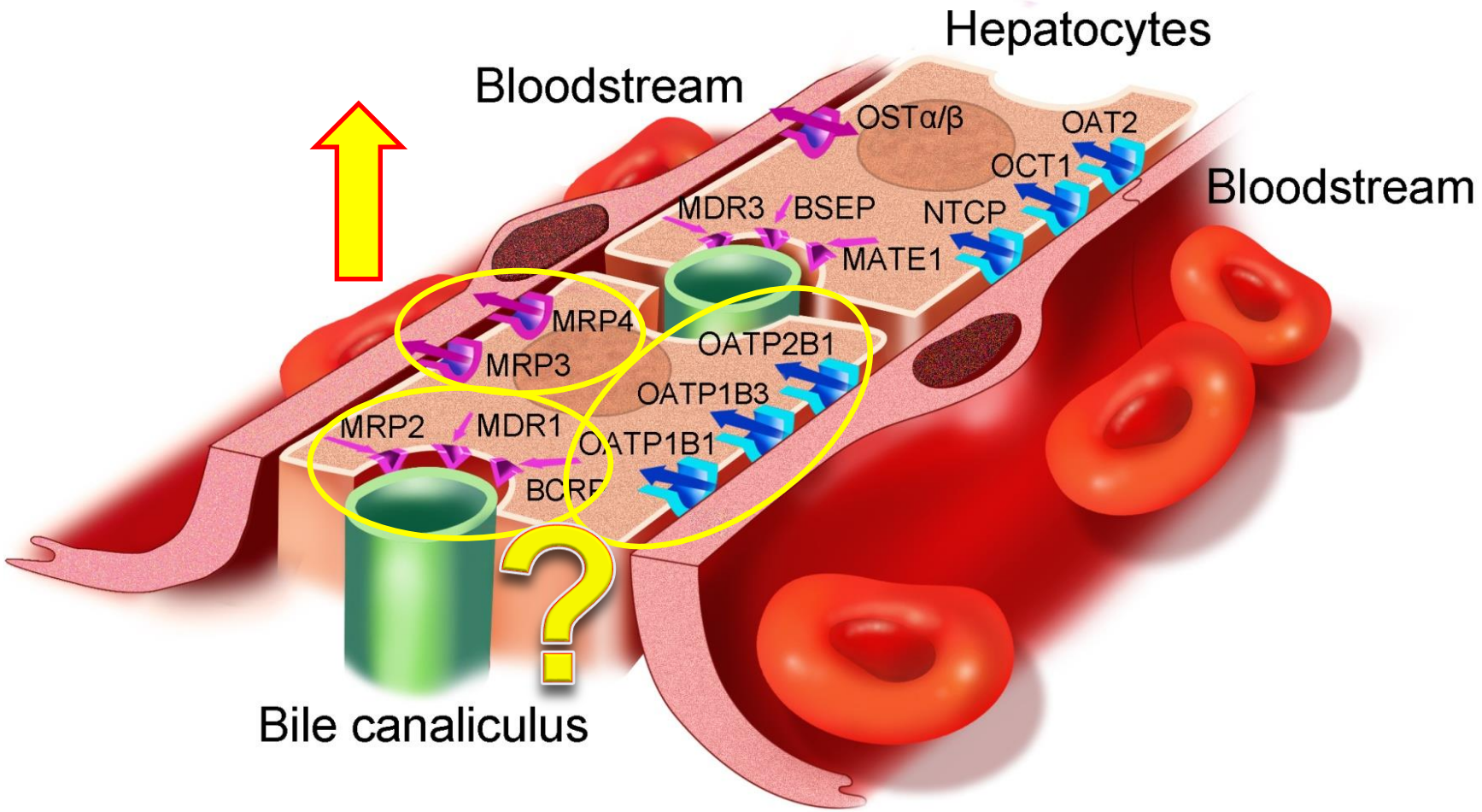






Hardwick et al., Drug Metab Dispos, **39**:2395, 2011

Impact of NASH-Mediated Changes in Hepatic Transporter Function on Systemic and Hepatic Drug Exposure



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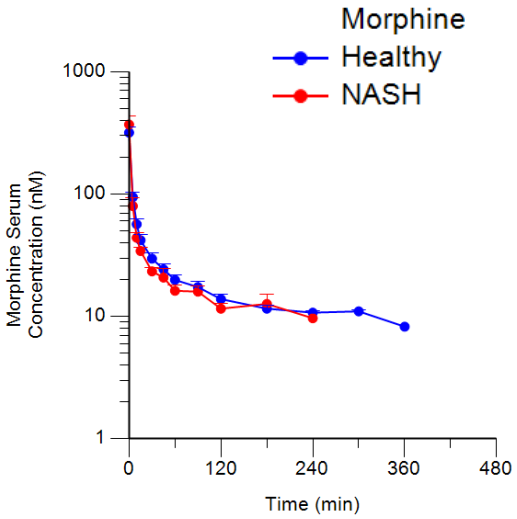
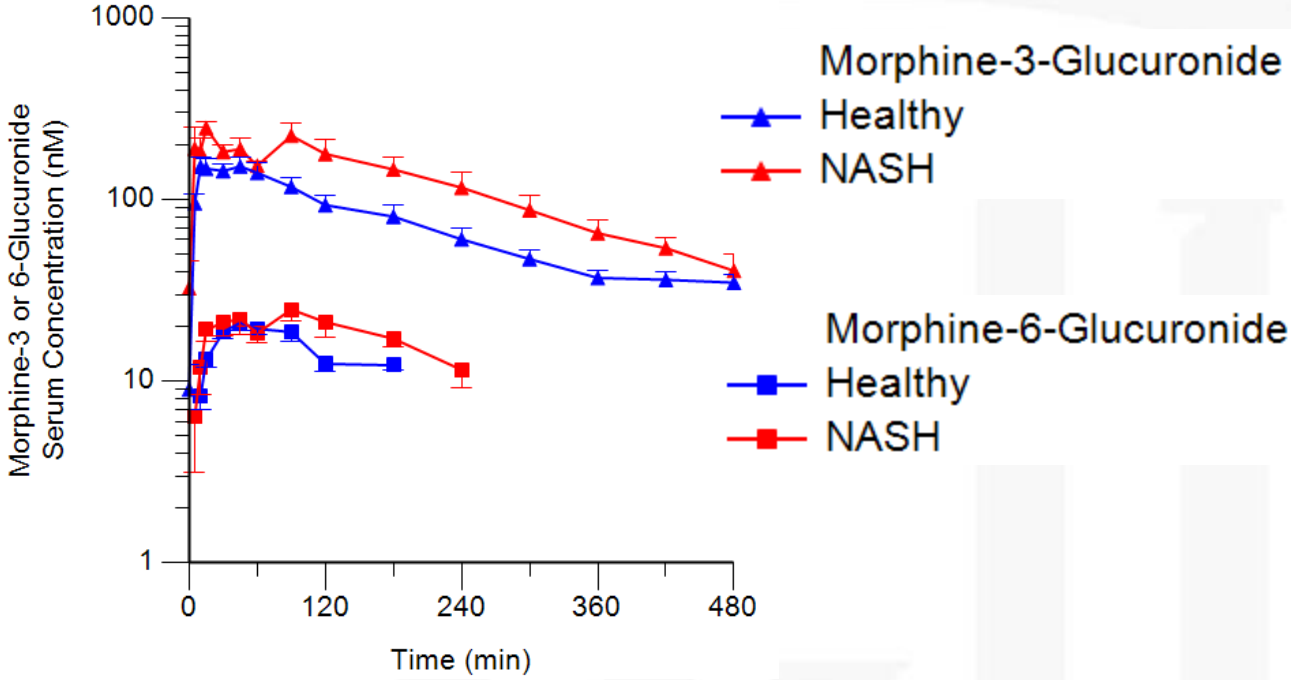
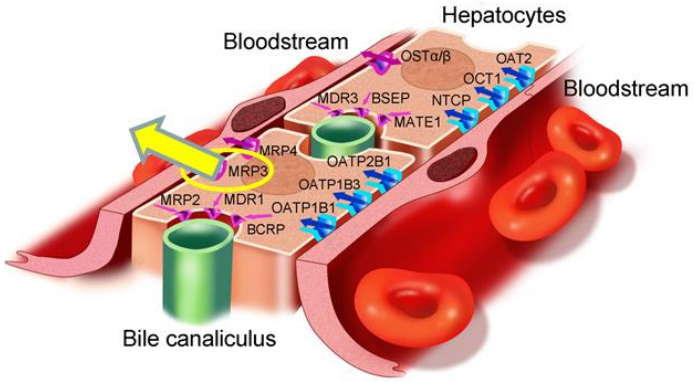
Increased M3G and M6G Serum Concentrations in NASH





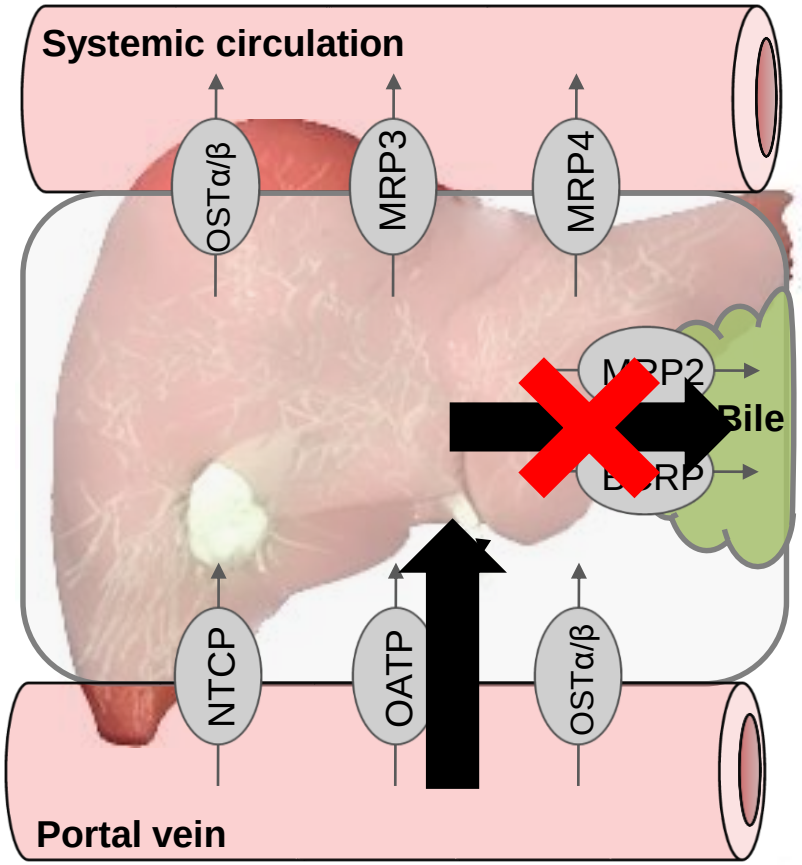


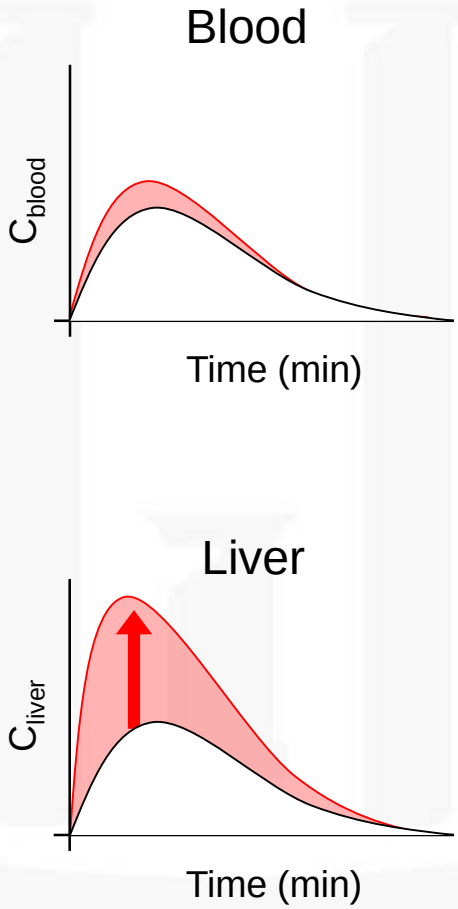
MG Parameters	Healthy (n=14)	NASH (n=7)
C_{max} (nM)	225 (194-261)	343** (284-413)
$AUC_{0\text{-last}}$ (μM*min)	37 (32-44)	59 ** (42-83)
Half-life (min)	187 (153-229)	146 (104-205)

Geometric mean (95% CI); ** p<0.01 *t*-test on log transformed data

Ferslew…Brouwer, Clin Pharmacol Ther, **97**:419, 2015

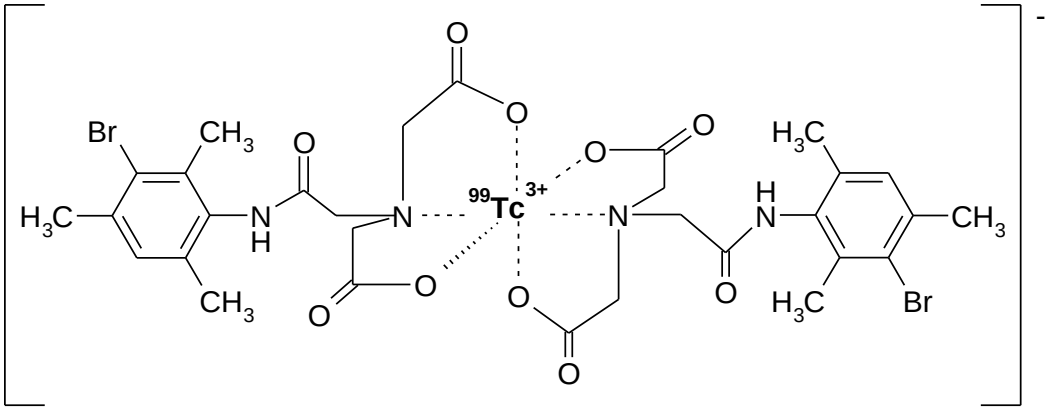
Simulations Predict That MRP2 Substrates Have Increased Hepatic Exposure in NASH





Köck and Brouwer, Clin Pharmacol Ther, **92**:599, 2012

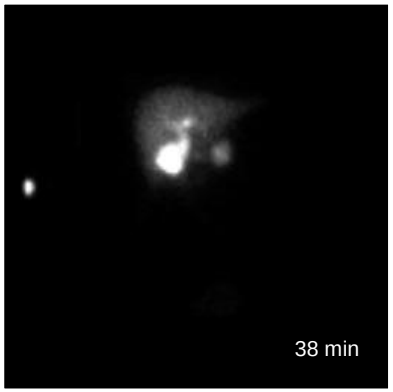
^{99m}Tc-Mebrofenin (Choletec®):
Probe for Transporter-Mediated Hepatobiliary Excretion

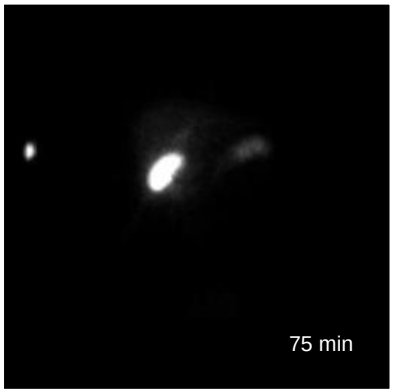


- Used clinically as a hepatobiliary imaging agent
- Liver uptake ~98%; negligible metabolism
- Urinary excretion <2% of dose
- Transporter-mediated hepatobiliary disposition
  - Hepatic uptake via OATP1B1 and OATP1B3
  - Biliary excretion via MRP2
  - Basolateral excretion via MRP3

Ghibellinii…Brouwer, Pharm Res, **25**:1851, 2008

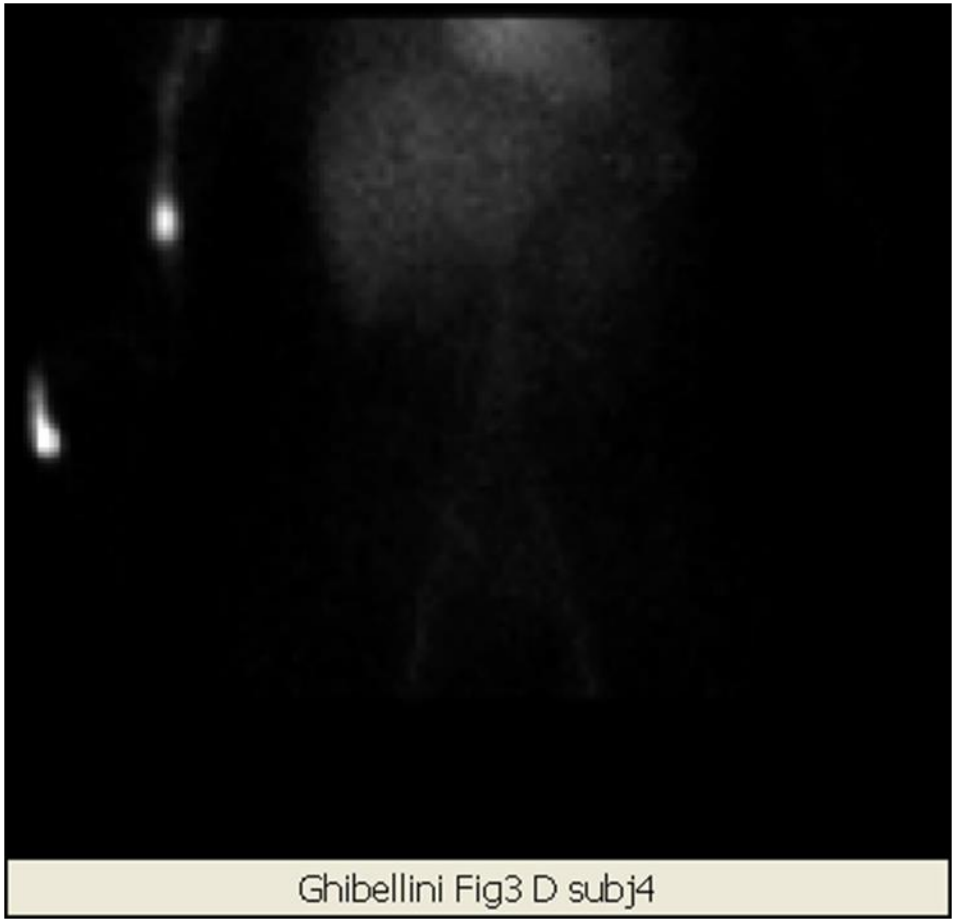
Gamma Scintigraphic Images (0-180 min) of ^{99m}Tc-Mebrofenin Hepatic Disposition





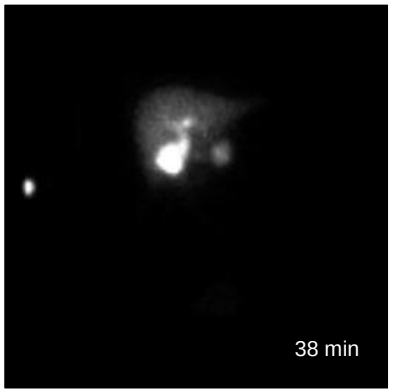
- ^{99m}Tc-mebrofenin rapidly distributes into the liver , is excreted into bile, and collects in the gall bladder

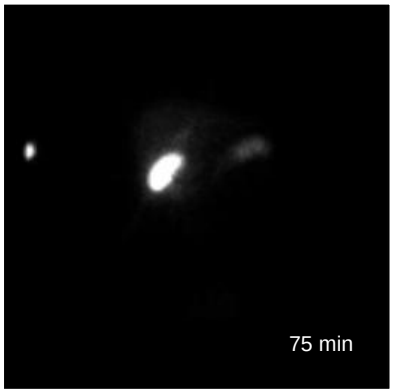
- Liver t_{max} ~13min



Ghibellini et al. AAPS Journal **6** (4) Article 33, 2004

Gamma Scintigraphic Images (0-180 min) of ^{99m}Tc-Mebrofenin Hepatic Disposition





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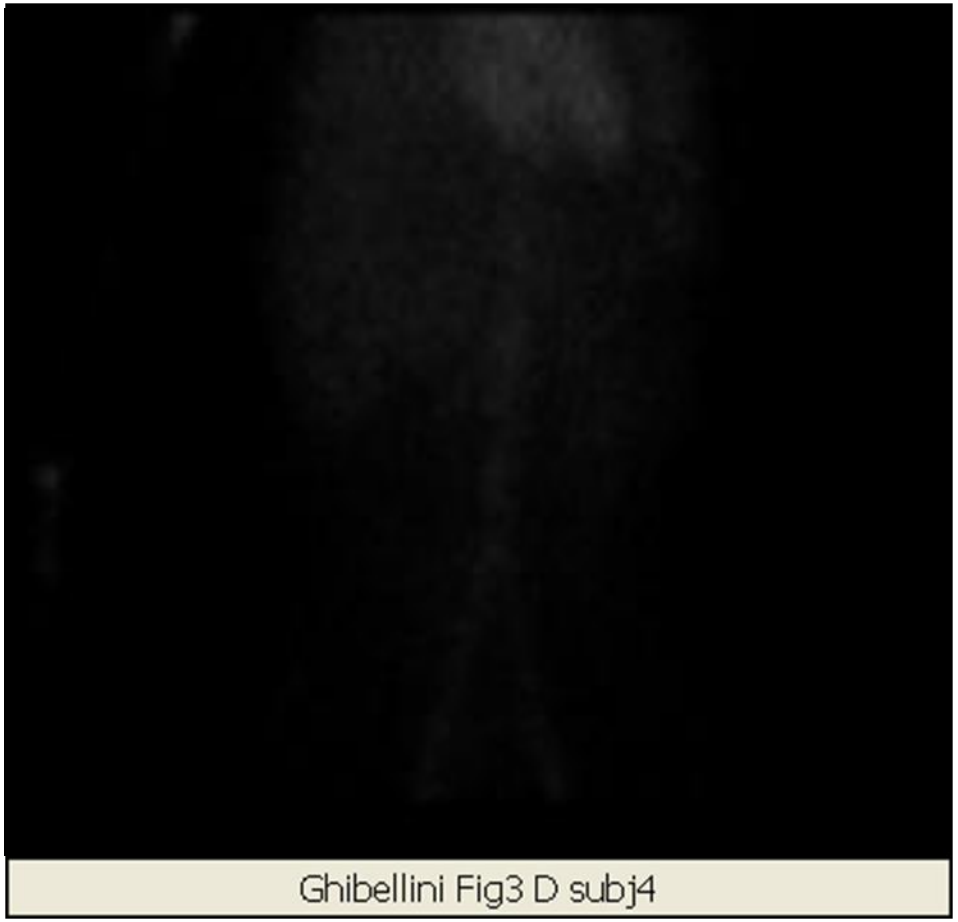


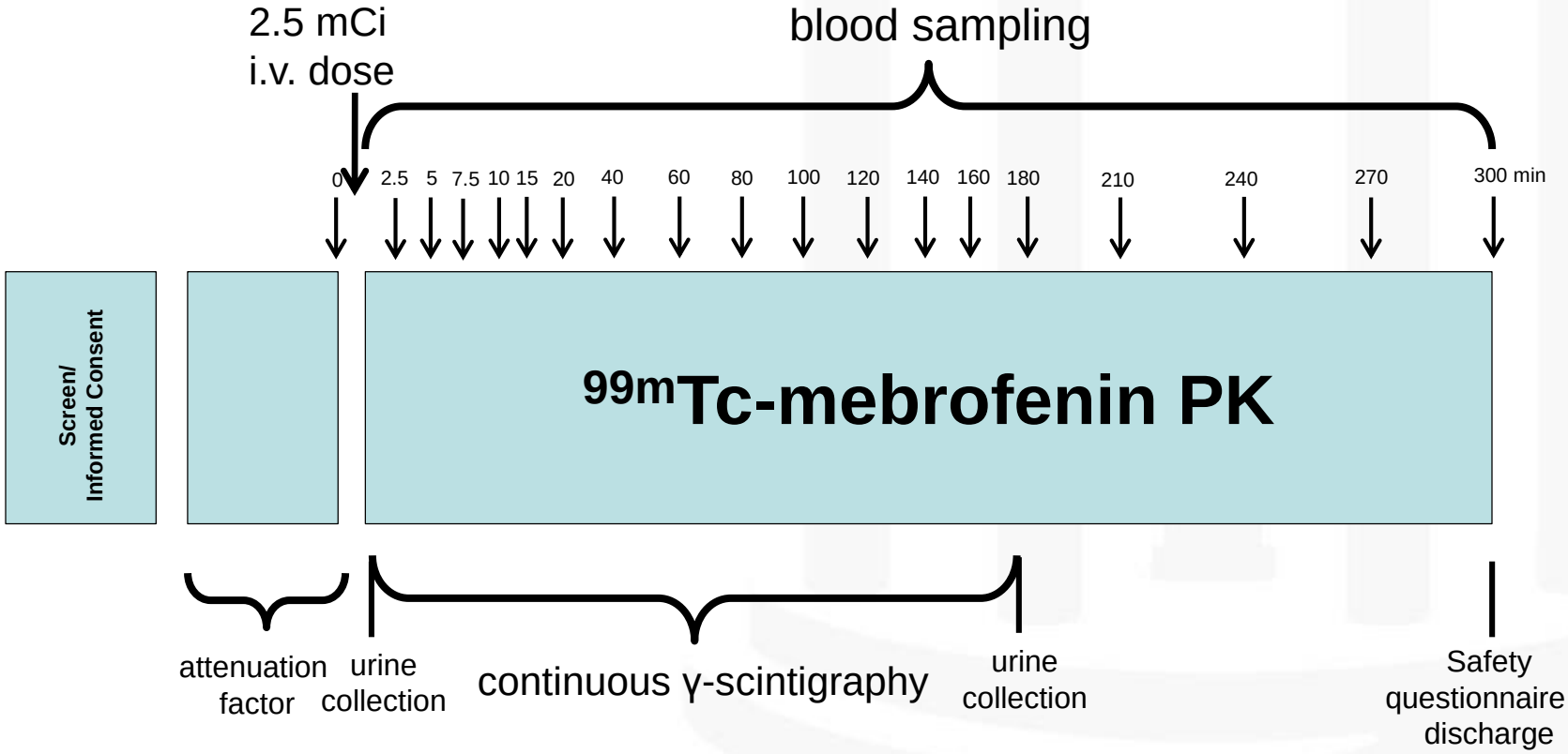
Ghibellini et al. AAPS Journal **6** (4) Article 33, 2004

Study Objectives

- Determine the systemic and hepatic exposure of ^{99m}Tc-mebrofenin, an organic anion transporter probe, in patients with biopsy-confirmed NASH compared to healthy subjects
- Utilize a pharmacokinetic model describing the systemic and hepatic disposition of ^{99m}Tc-mebrofenin to evaluate NASH-mediated alterations in hepatic transporter function

Clinical Study Design

- Subjects admitted on morning of study after an overnight fast
- Attenuation correction obtained with a cobalt-57 flood source
- Subjects positioned supine under gamma camera



- Subjects discharged following exit exam

Demographics and Clinical Chemistries

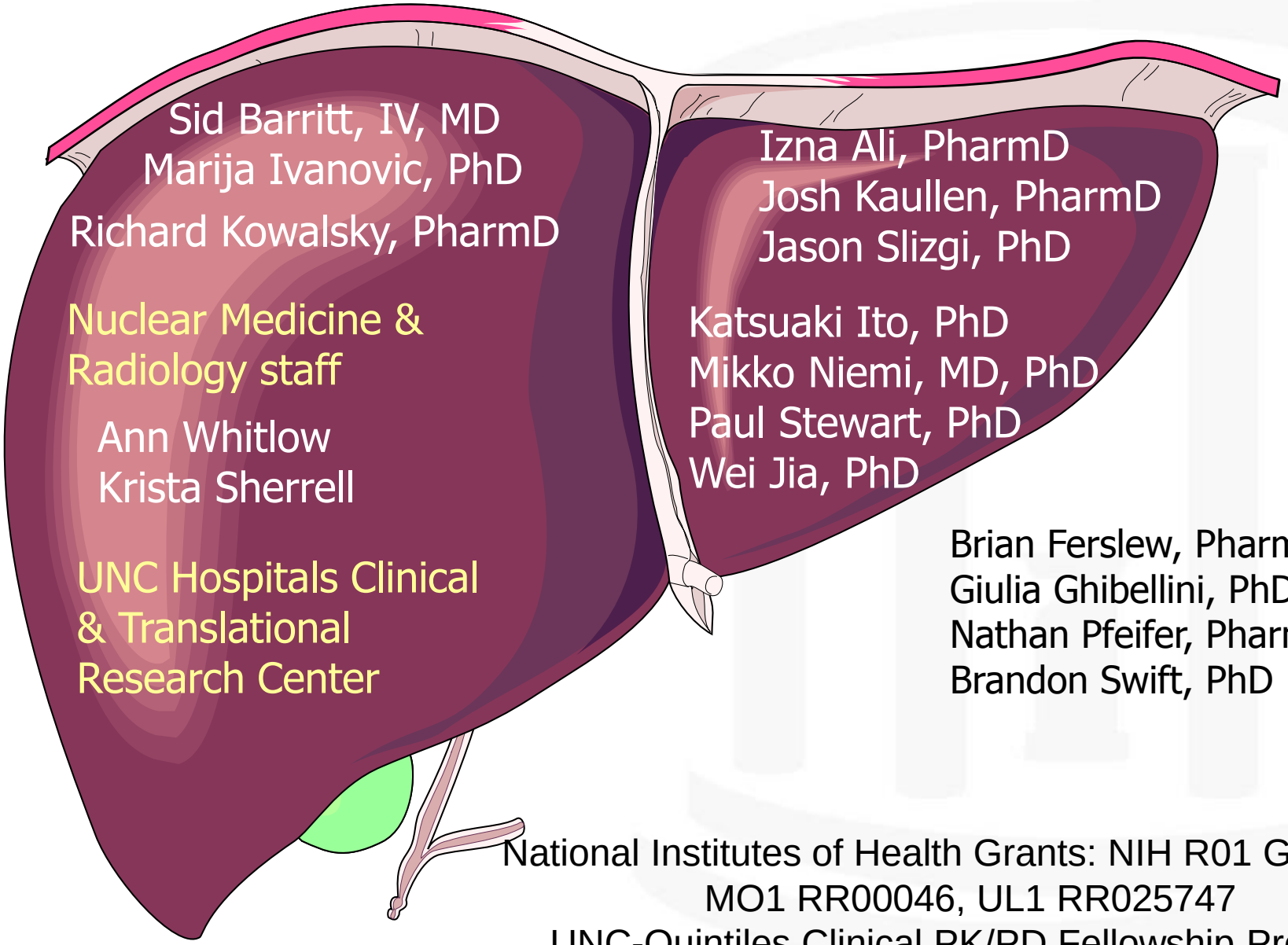
	Control (n=14)	NASH (n=7)
Gender	8 M; 6 F	4 M; 3 F
Ethnicity	14 non-Hispanic	1 Hispanic; 6 Non-Hispanic
Race	11 Caucasian; 3 African-American	5 Caucasian; 1 Mexican; 1 Asian
Age (yrs)	38.9 ± 15.4	37.4 ± 17.4
Body Weight (kg)	72.1 ± 12.1	102 ± 16*
BMI (kg/m^2)	24.4 ± 2.2	33.3 ± 5.1*
Creatinine (mg/dL)	0.86 ± 0.17	0.83 ± 0.15
Bilirubin, total (mg/dL)	0.729 ± 0.237	0.957 ± 0.391
Albumin (g/dL)	4.20 ± 0.20	4.49 ± 0.38
ALT (u/L)	28.7 ± 9.8	113 ± 60*
AST (u/L)	25.2 ± 8.0	72.9 ± 34.3*
HOMA-IR	1.56 ± 0.53	8.18 ± 4.56*
ALP (u/L)	56.3 ± 17.8	68.1 ± 20.0

Mean ± SD; *p < 0.05 using 2-tailed Student's *t*-test

Summary

- Hepatic transport protein expression and function are altered in patients with NASH, which may impact the systemic and/or hepatic exposure to substrates [drugs, metabolites, and endogenous compounds (*e.g.,* bile acids)]
- **Impaired MRP2 function**
  - ^{99m}Tc-Mebrofenin ***hepatic*** and systemic exposure were significantly increased in NASH
- **MRP3 upregulation**
  - Morphine glucuronide ***systemic*** exposure (C_{max}, AUC) and conjugated bile acid serum concentrations were significantly associated with NASH severity
- Patients with NASH have higher fasting and post-prandial exposure to bile acids, including the more hydrophobic and cytotoxic species. Bile acid profiles may be useful in the diagnosis of NASH.

Acknowledgements



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