

# PNPLA3 pathobiology for NASH implications therapeutics

*9<sup>th</sup> Paris MASH meeting  
7<sup>th</sup>-8<sup>th</sup> September 2023*

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UNIVERSITY OF GOTHENBURG  
THE SAHLGRENKA ACADEMY

**SR has consulted in the field of MASH for AstraZeneca, Wave-life science, Ribocure and Foresite Labs**

**SR has a basic science research grant provided by Astra Zeneca**

# Natural history of Steatotic Liver Disease

## Quantity:

Excess in food intake, lack of exercise

## Quality:

Fructose  
Saturated fat  
Alcohol

Normal Liver

Steatosis

Genetic susceptibility

Common Variants

*PNPLA3*

*TM6SF2*

*MBOAT7*

*GCKR*

*HSD17B13*

*MARC1*

*APOE*

*PSD3*

*GPAM, ATG7*

Rare Variants

*ATG7*

*APOB*

*CIDEA*

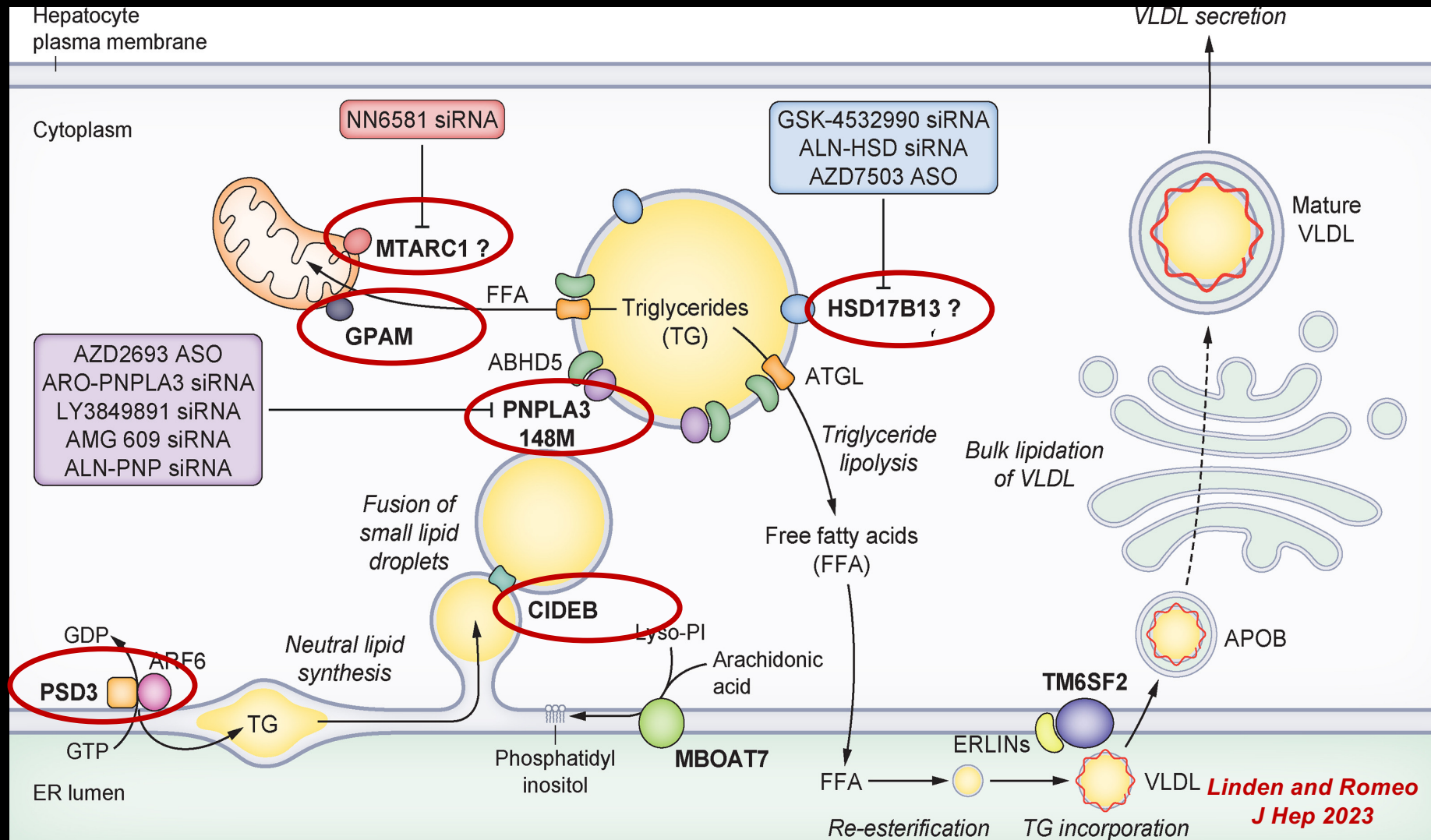
Steato-Hepatitis

Cirrhosis

Hepatocellular  
Carcinoma

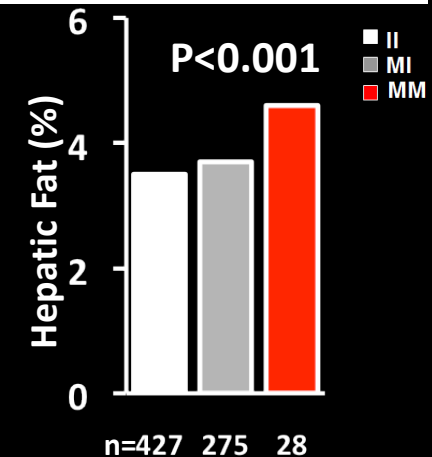
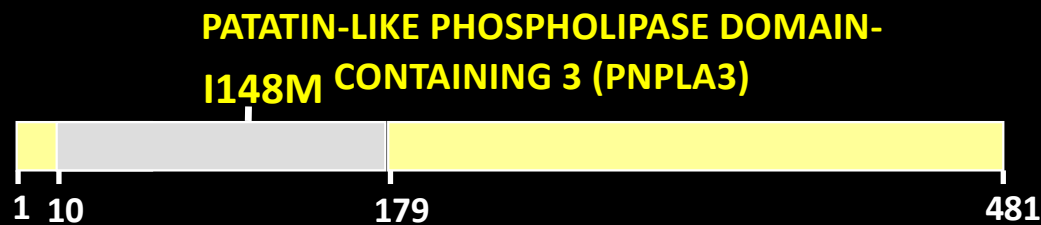
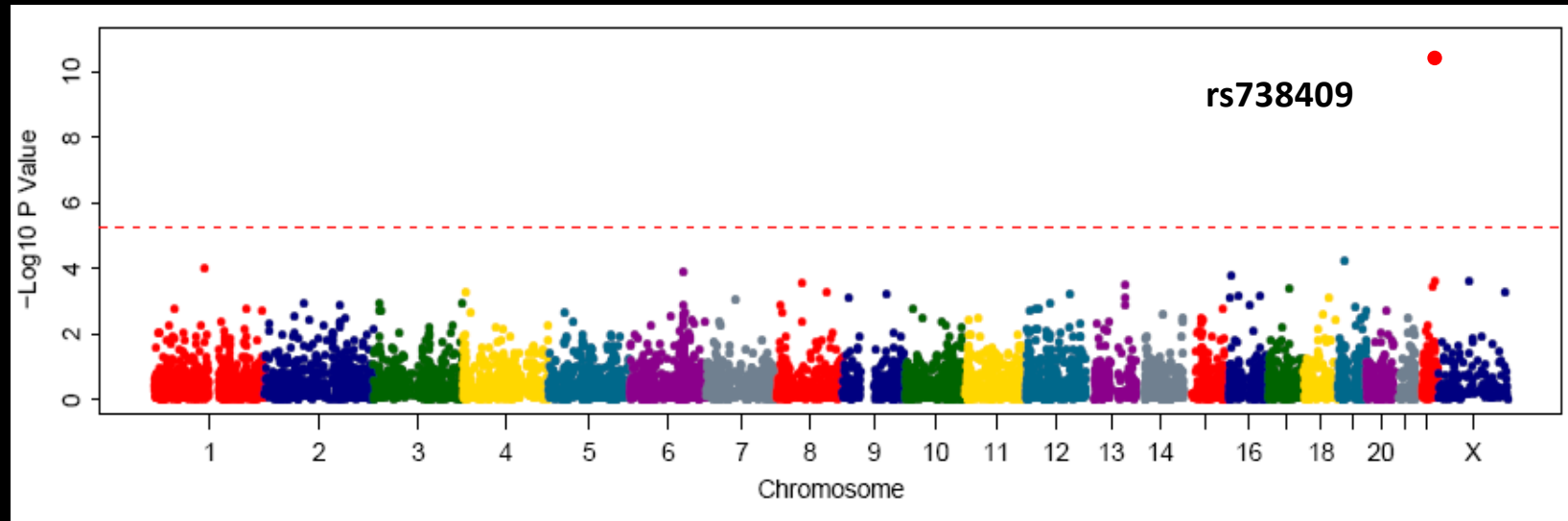
S Romeo Cell Met 2020

# Therapeutic Targets identified by human genetics

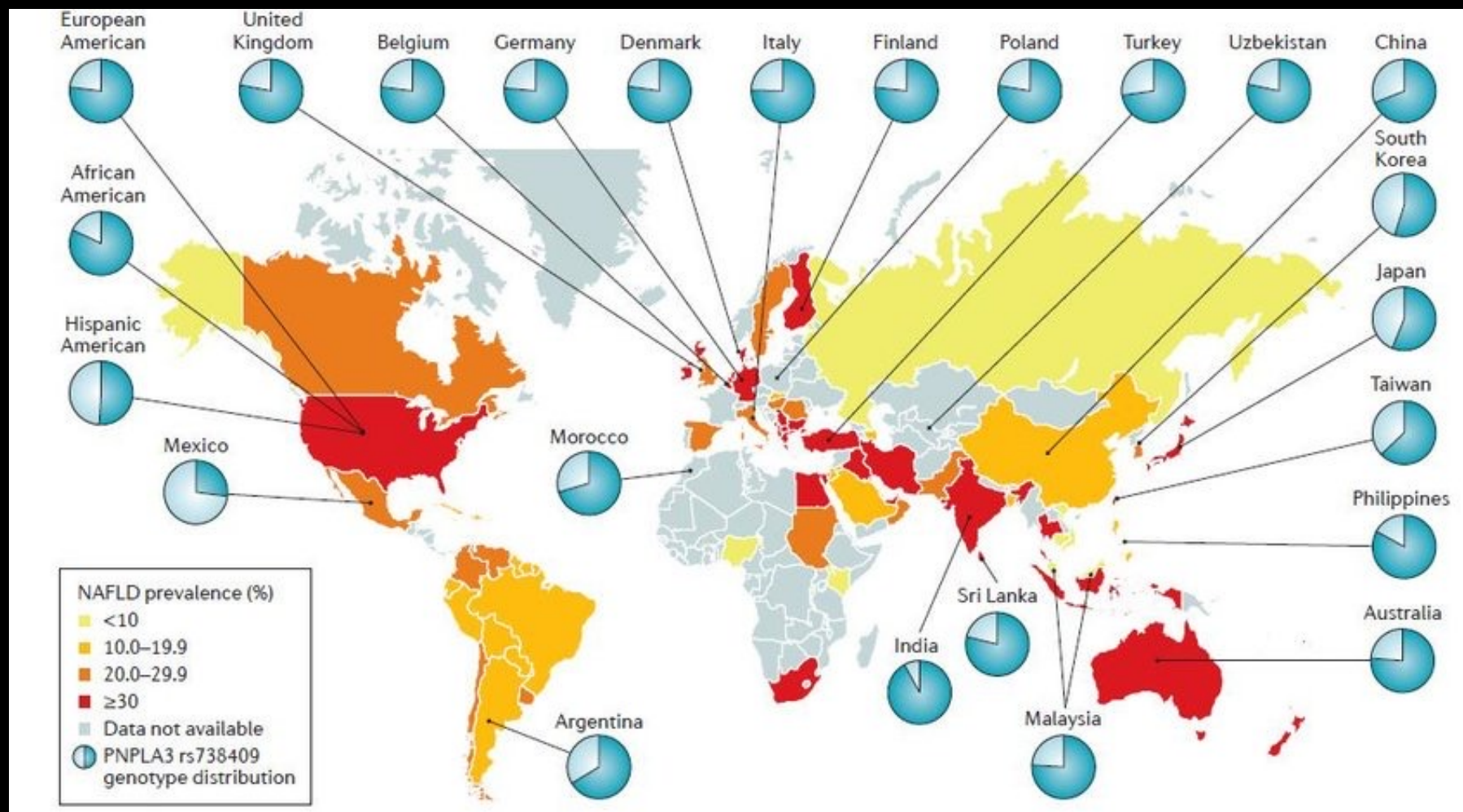


***What is the role of PNPLA3 in MASH?***

# **PNPLA3 gene associates with increased liver fat content**

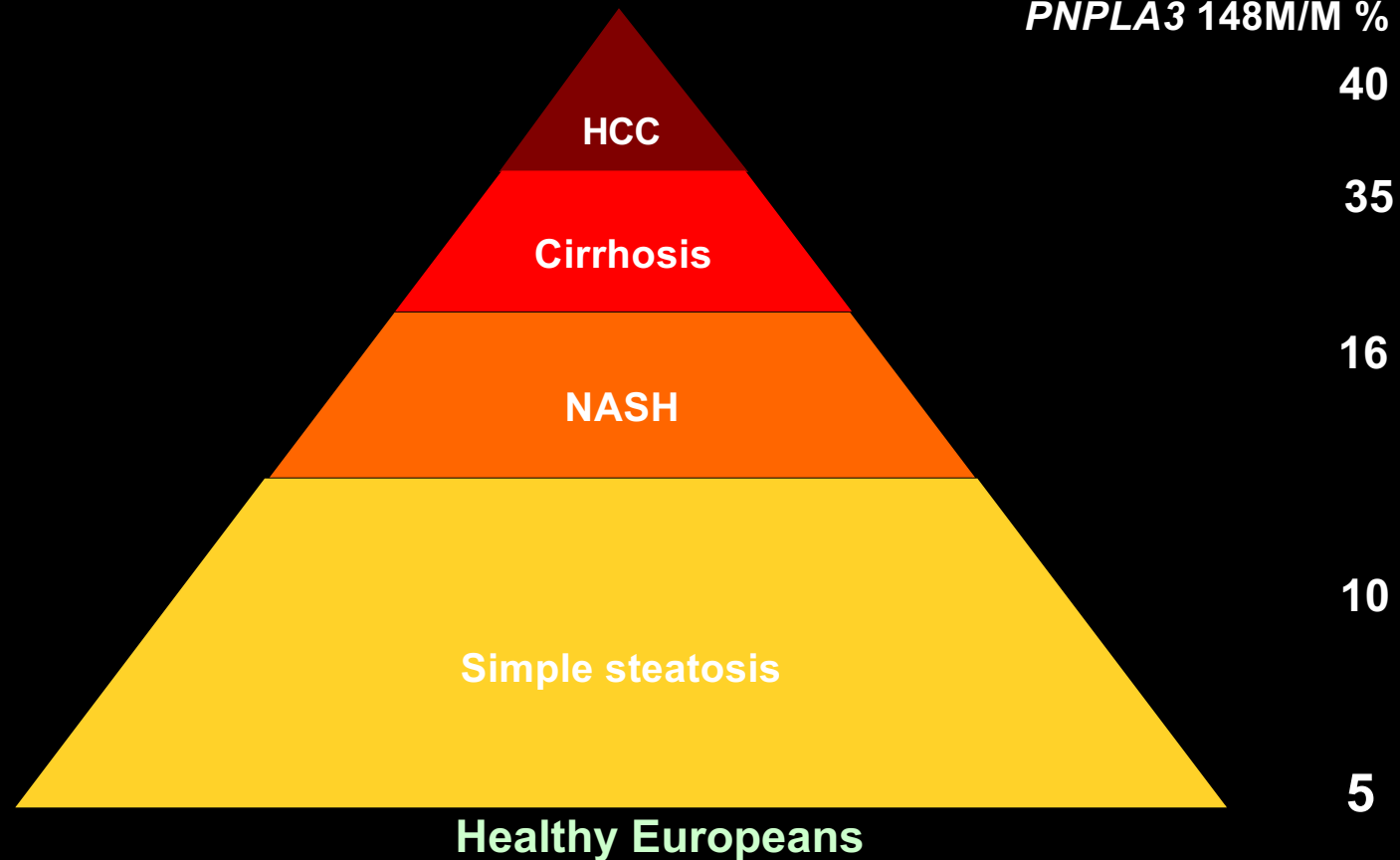


# ***PNPLA3 rs738409 Frequency in the General Population***



Disease	Risk PNPLA3 M148M <sup>1</sup>
Liver disease OR	N=5,100 <b>3.3</b> (95% CI 2.7-3.9)
HCC OR	N=100 <b>12.2</b> (95% 6.9-21.6)

<sup>1</sup>Liu et al J Hep 2014

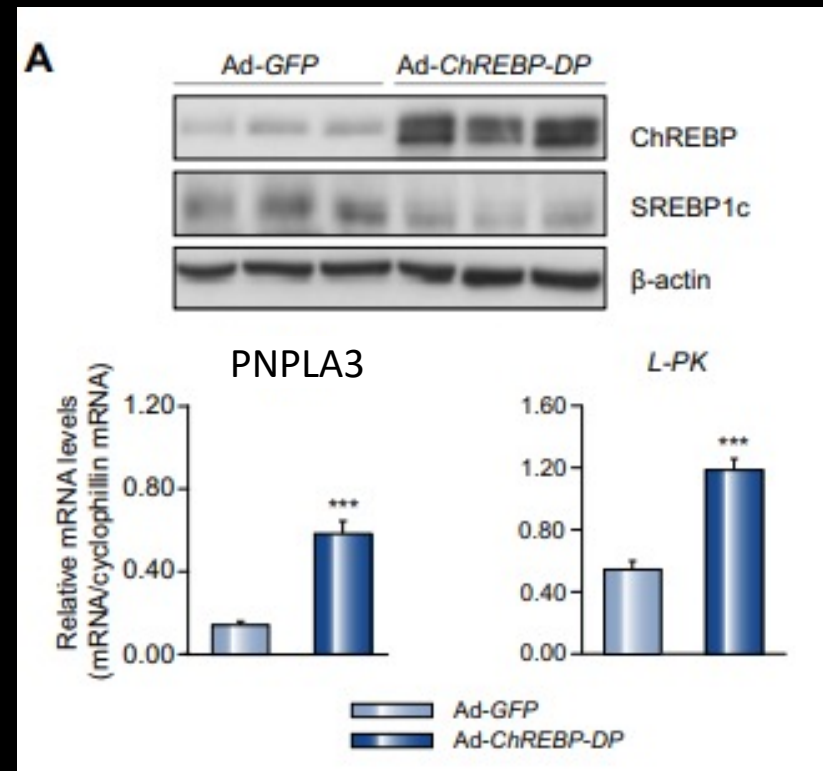
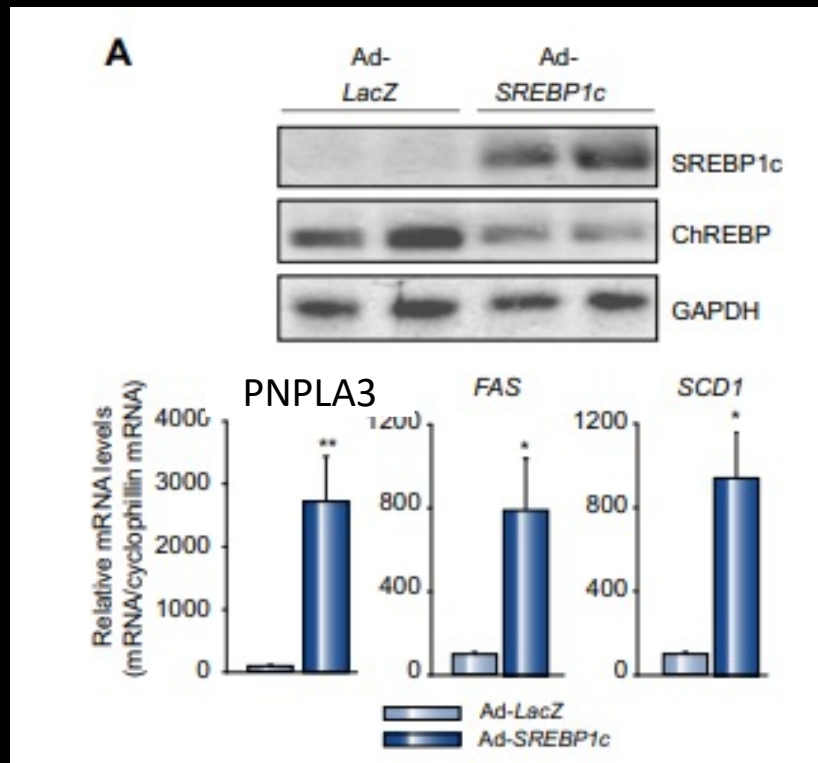




***Is PNPLA3 upregulated in MASH?***

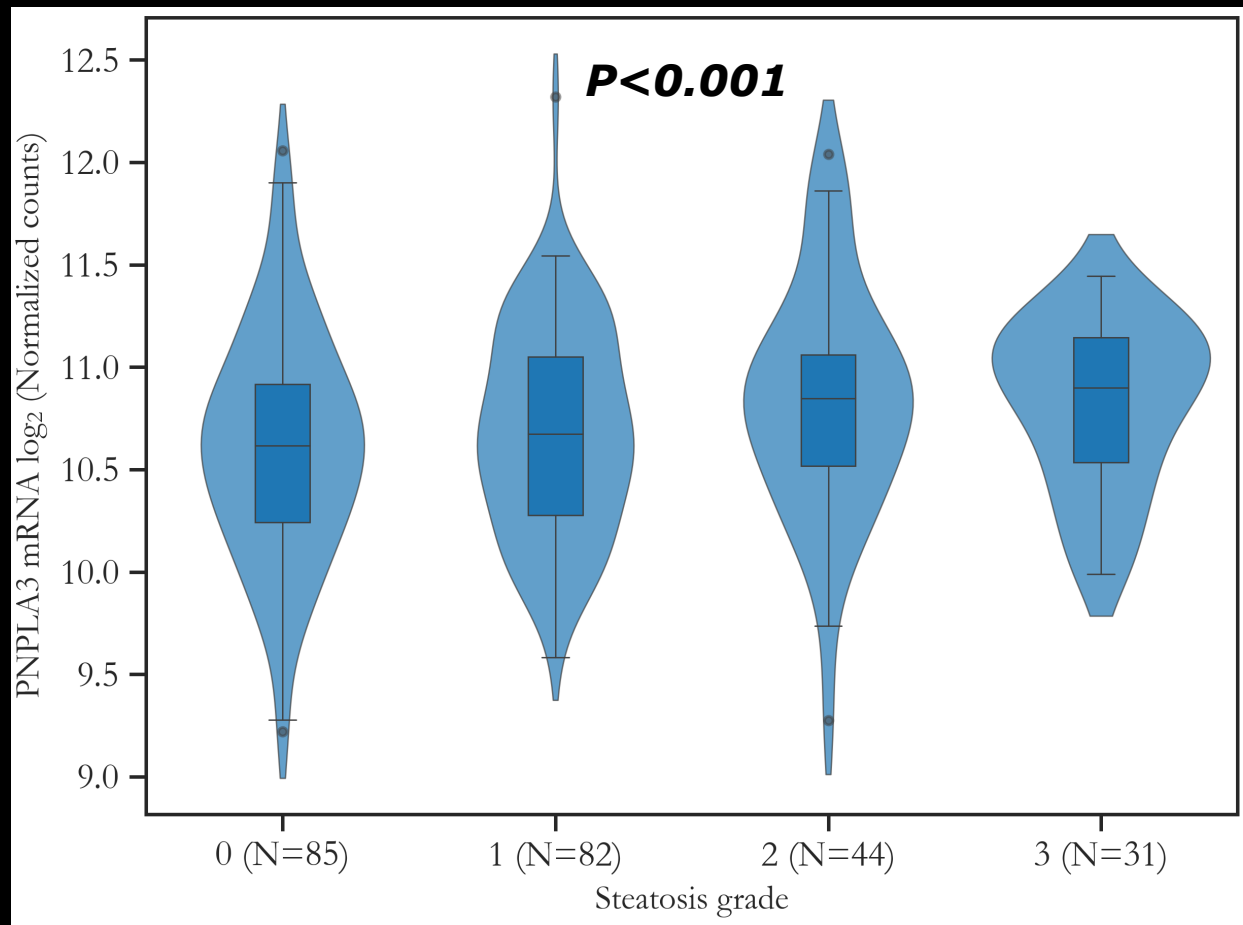
***YES!***

# PNPLA3 is upregulated by SREBP1C and CHREBP



*Dubuquoy et al J Hep 2011*

# PNPLA3 mRNA expression increases with hepatic steatosis grades



**Unpublished data**

PNPLA3 is highly expressed in 2 liver cell types

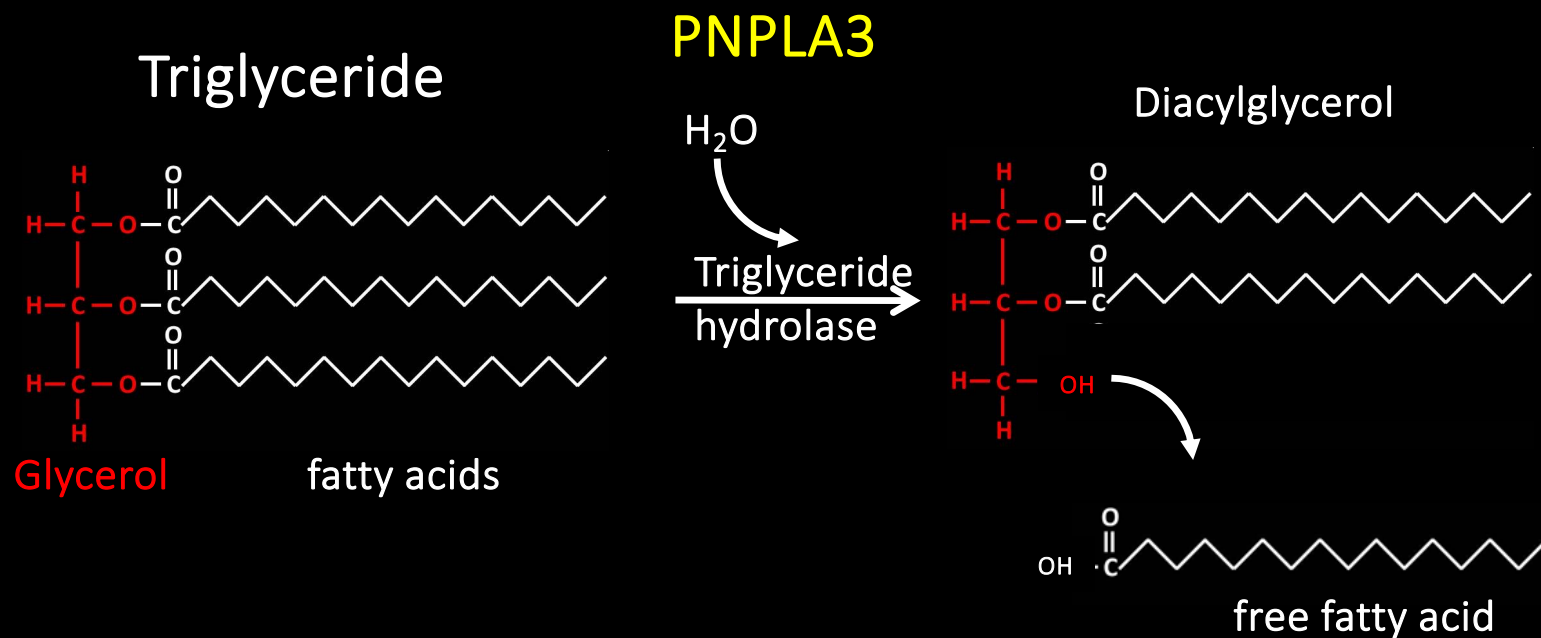
Hepatocyte



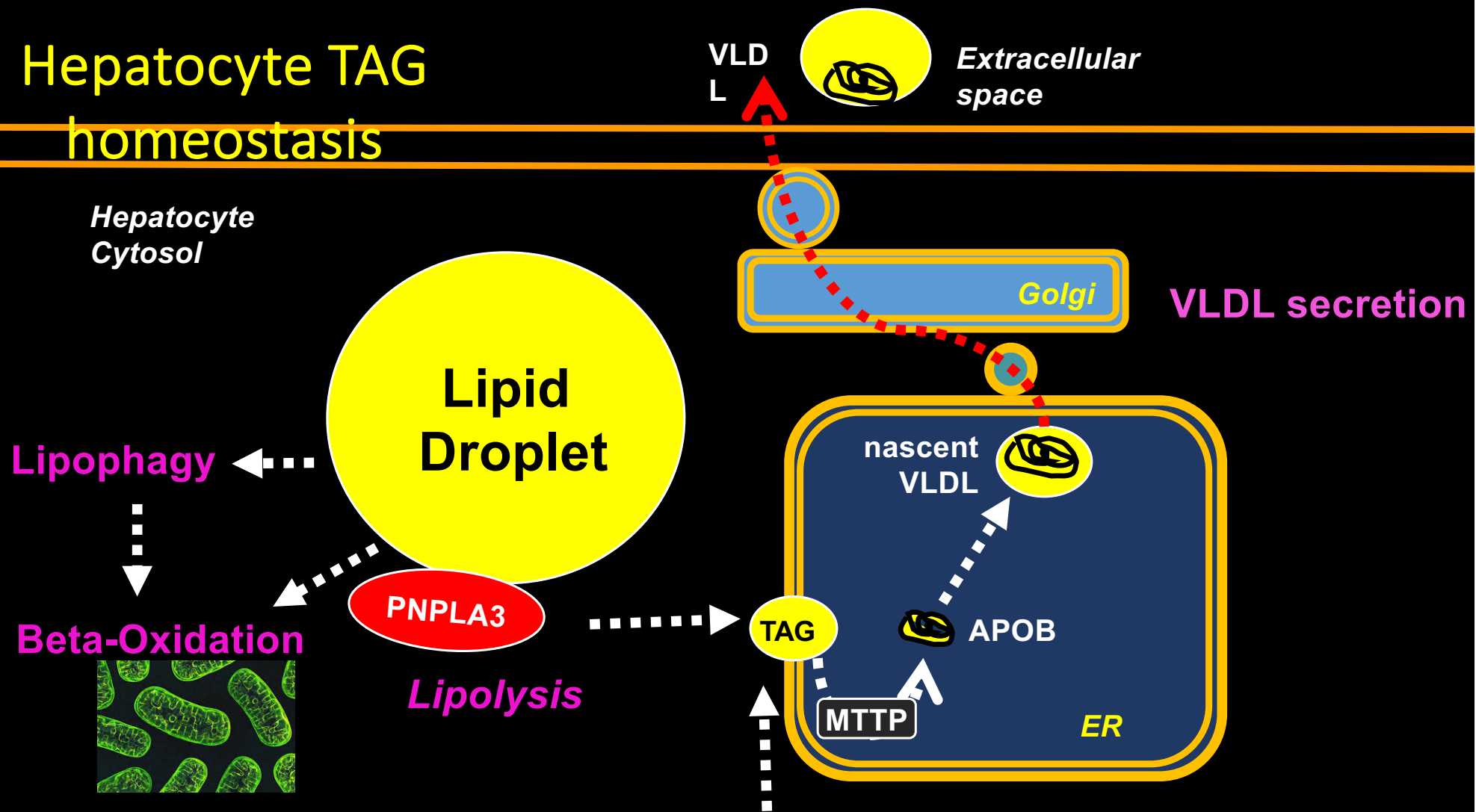
Hepatic Stellate Cells



# PNPLA3 has a triglyceride hydrolase activity



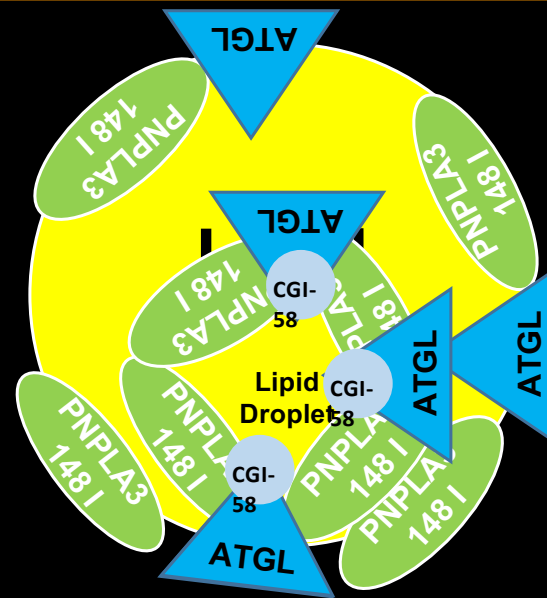
# Hepatocyte TAG homeostasis



TAG: triglyceride; ER: endoplasmic reticulum; MTTP: microsomal triglyceride transfer protein; APOB: apolipoprotein B; VLDL: very low density lipoprotein

*Extracellular  
space*

*Cytosol*

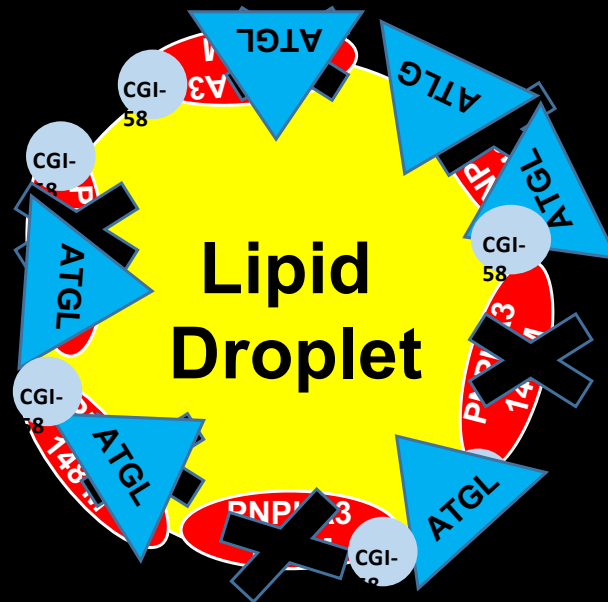


*PNPLA3=ADIPONUTRIN*  
*ATGL=PNPLA2*

*Donati Hep 2015*  
*Pingitore Hum Mol Gen 2016*  
*Pirazzi Hum Mol Gen 2014*  
*Pirazzi J Hep 2012*  
*Wang Hep 2019*

*Extracellular  
space*

*Cytosol*



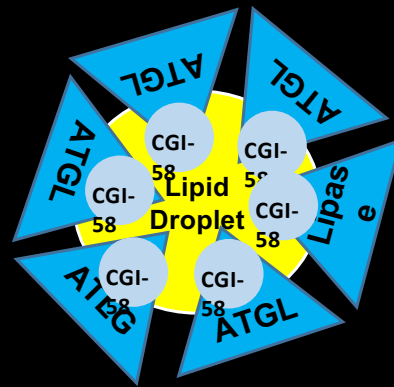
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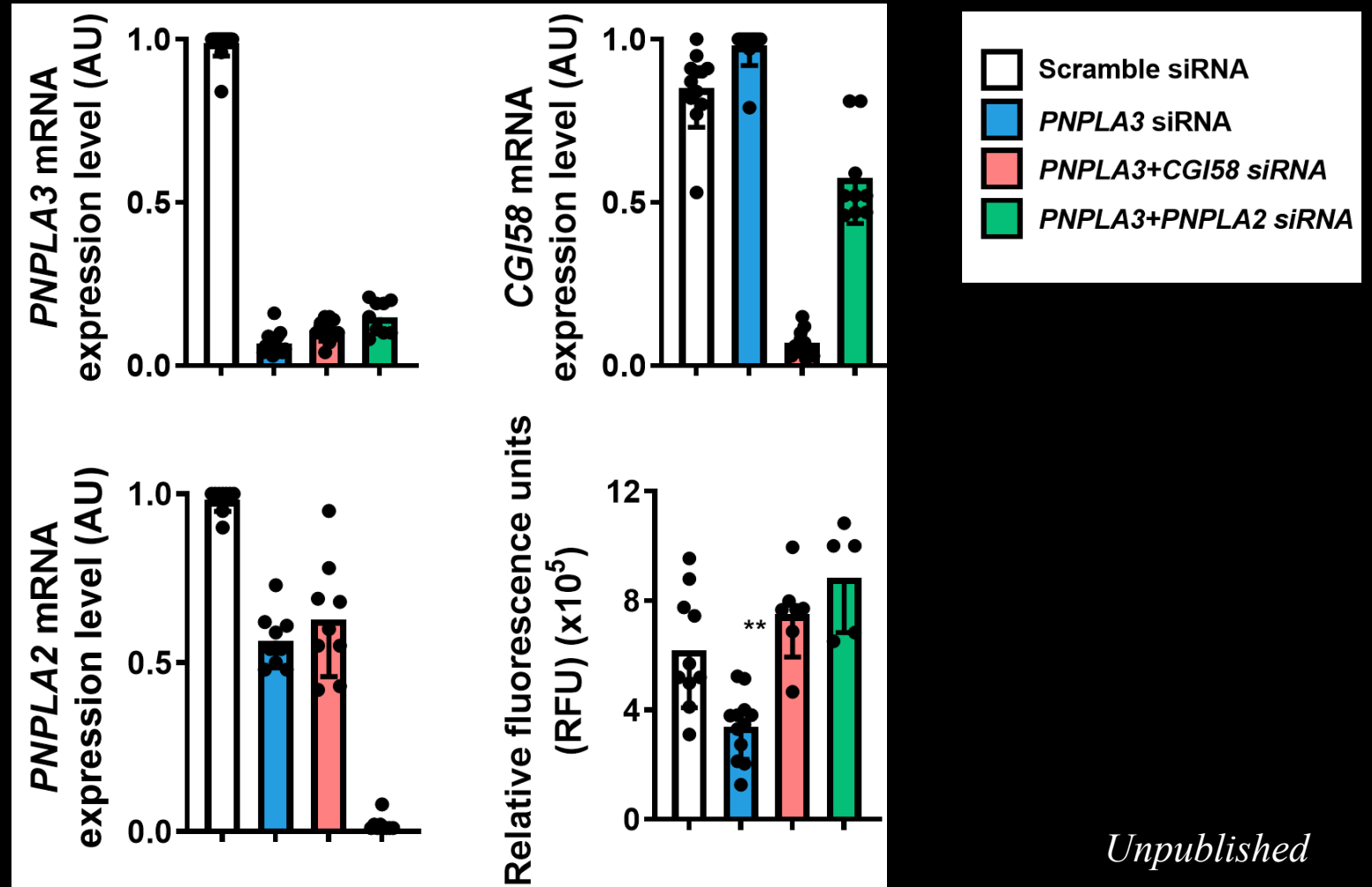
*Extracellular  
space*

*Cytosol*



*PNPLA3=ADIPONUTRIN*  
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## siPNPLA2/CGI58 abolish the beneficial siPNPLA3 effect in 3D HEPG2/LX2

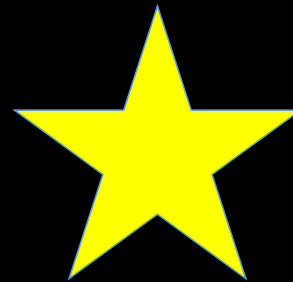


PNPLA3 is highly expressed in 2 liver cell types

Hepatocyte



Hepatic Stellate Cells



# Liver fibrosis is mediated by Hepatic Stellate Cells (HSC)

Hepatocyte  
damage



HSC activation (alpha sma)



ECM production (Collagen)



ECM Remodeling (MMPs, TIMPs)



Fibrosis

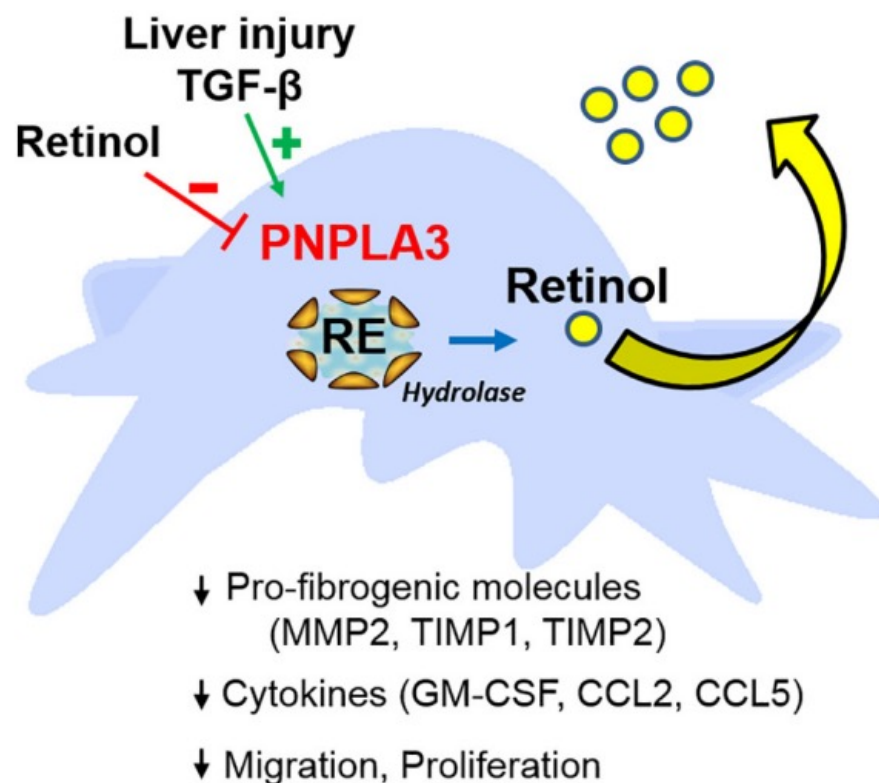
*ECM: extra cellular matrix*  
*MMP: metallo-protease*  
*TIMP: tissue inhibitors of MMP*

# Role of PNPLA3 in Hepatic Stellate Cells

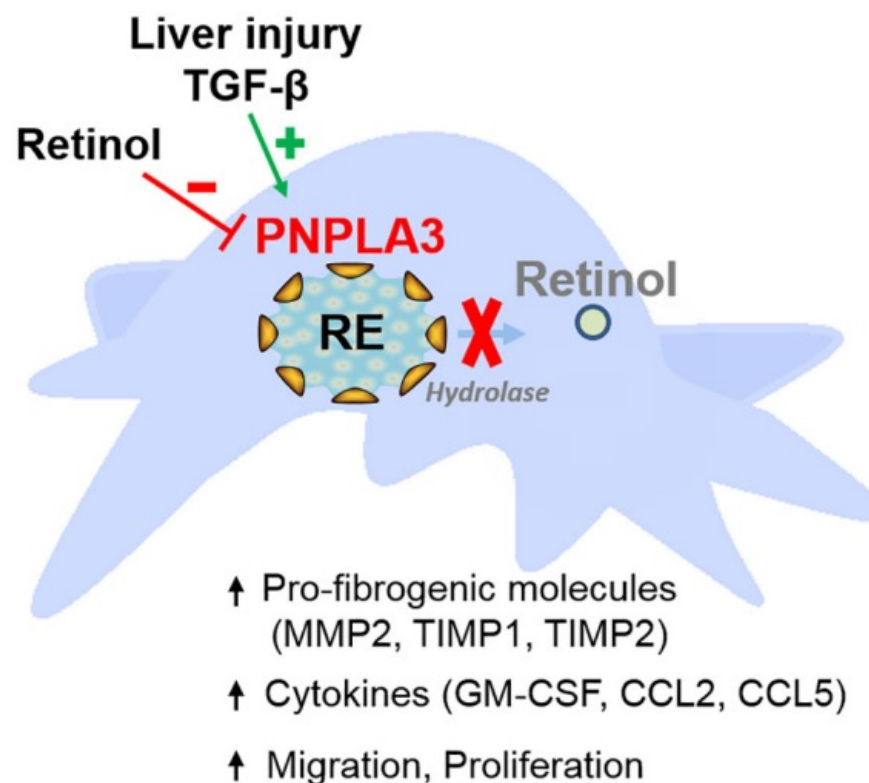
P. Pingitore, S. Romeo

BBA - Molecular and Cell Biology of Lipids 1864 (2019) 900–906

## PNPLA3 148I



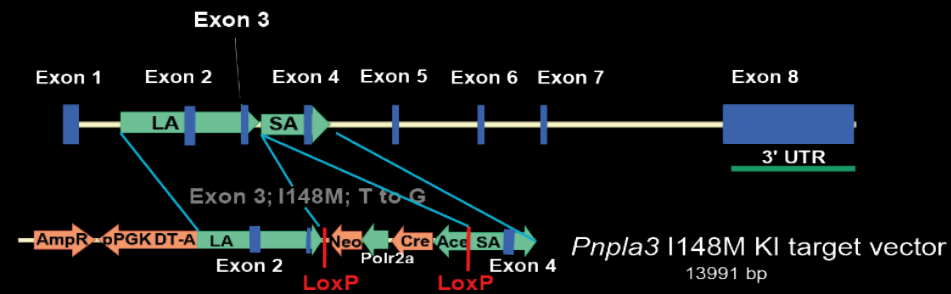
## PNPLA3 148M



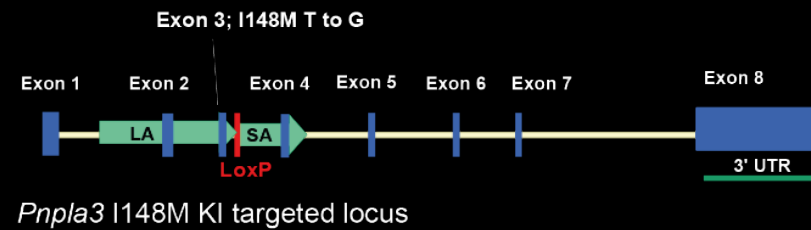
# PNPLA3 Knock In mouse I148M

## A

Mouse *Pnpla3* genomic locus



## B

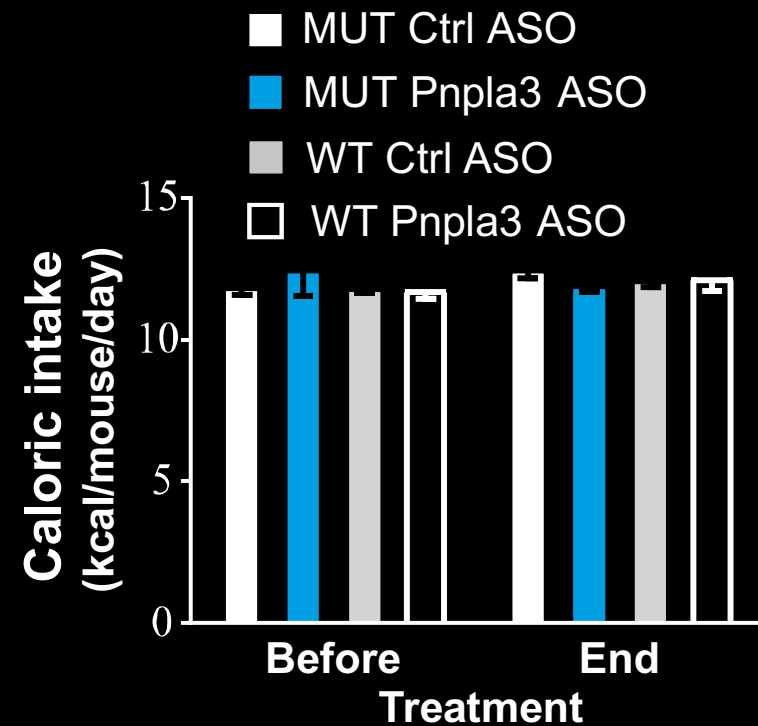
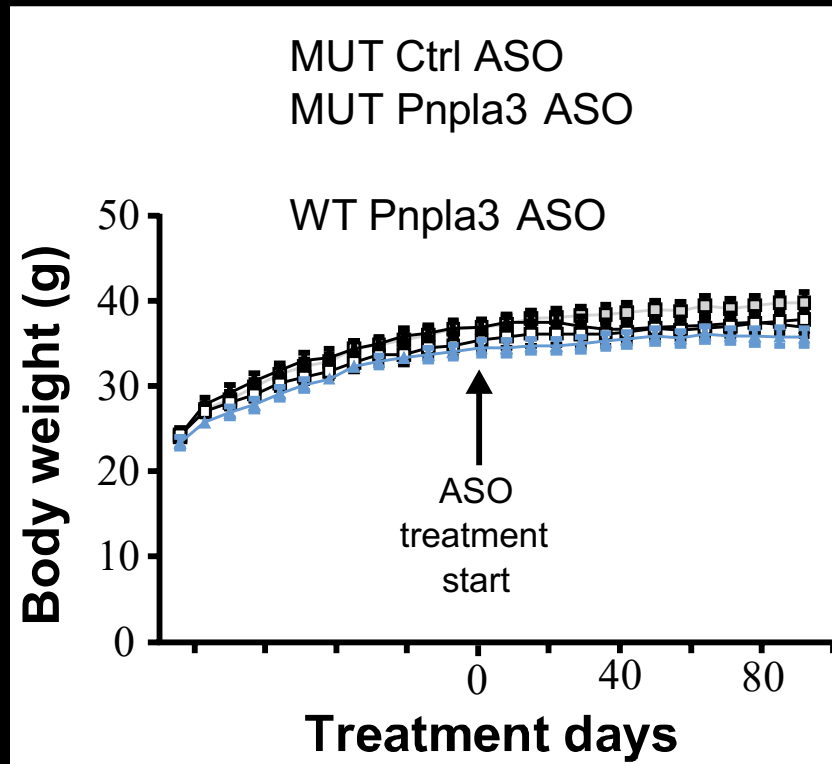


# Study design

- NASH-inducing\* diet 26 weeks
- Pnpla3 inhibition with ASO
- Effect on liver Steatosis, Inflammation and Fibrosis

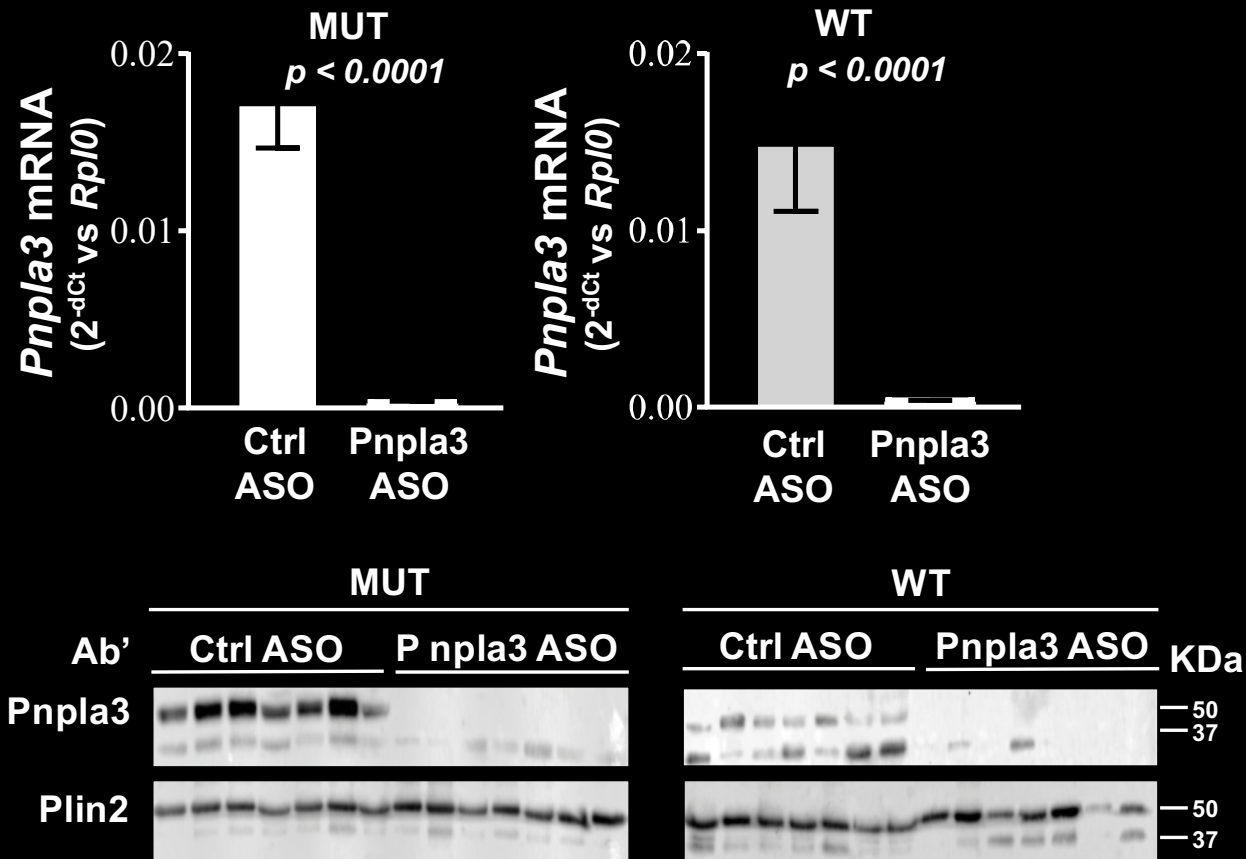
\* 40% fat (18% transfat), 40% Carbs (20% fructose), 2% cholesterol

## Pnpla3 ASO treatment did not change body weight

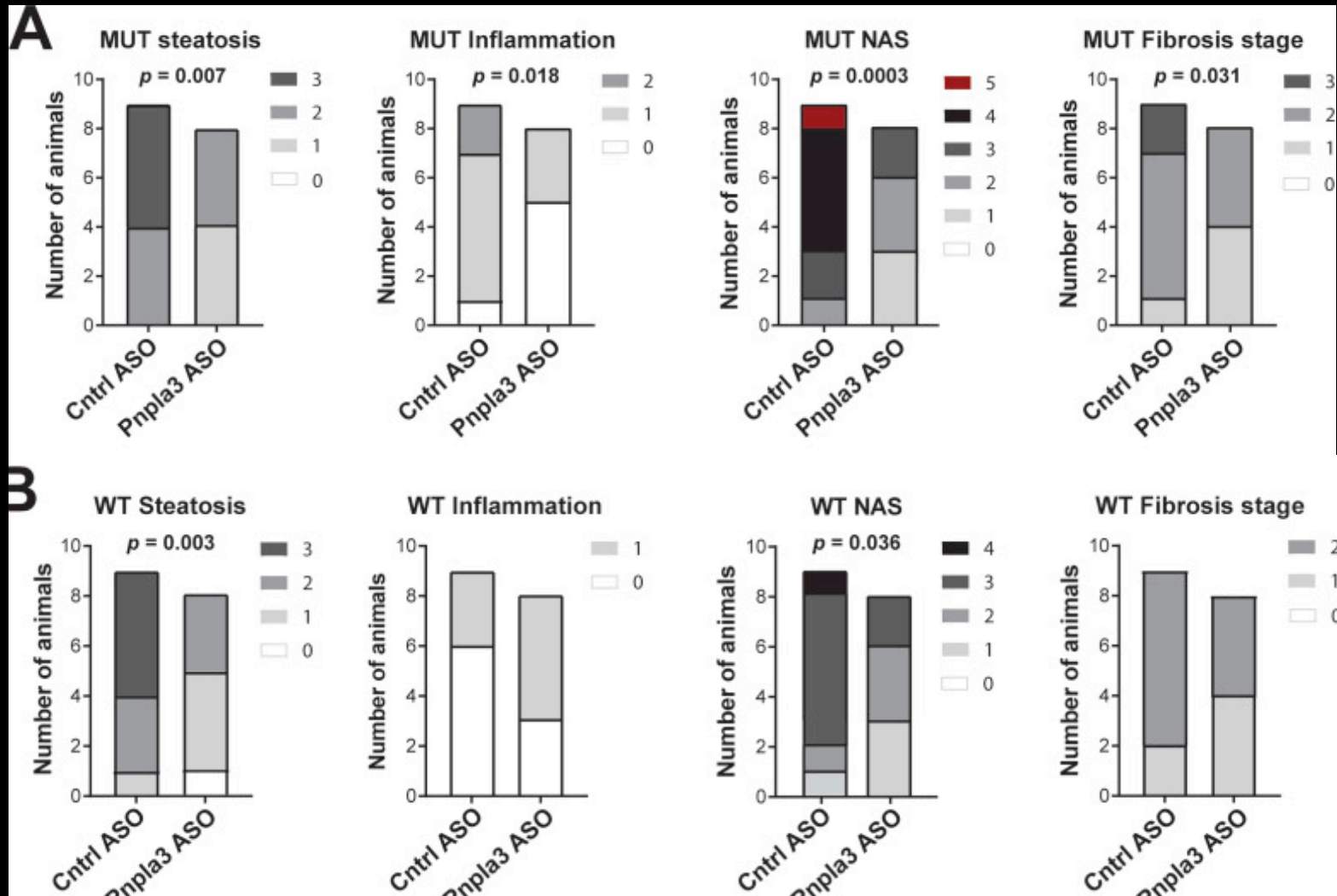




# ASO effectively reduced Pnpla3 expression and synthesis



# Effect of ASO-PNPLA3 on liver histology



## Overall effect on MASLD component

Pnpla3 Inhibition	MUT	WT
Steatosis	↓	↓
Inflammation	↓	
Fibrosis	↓	

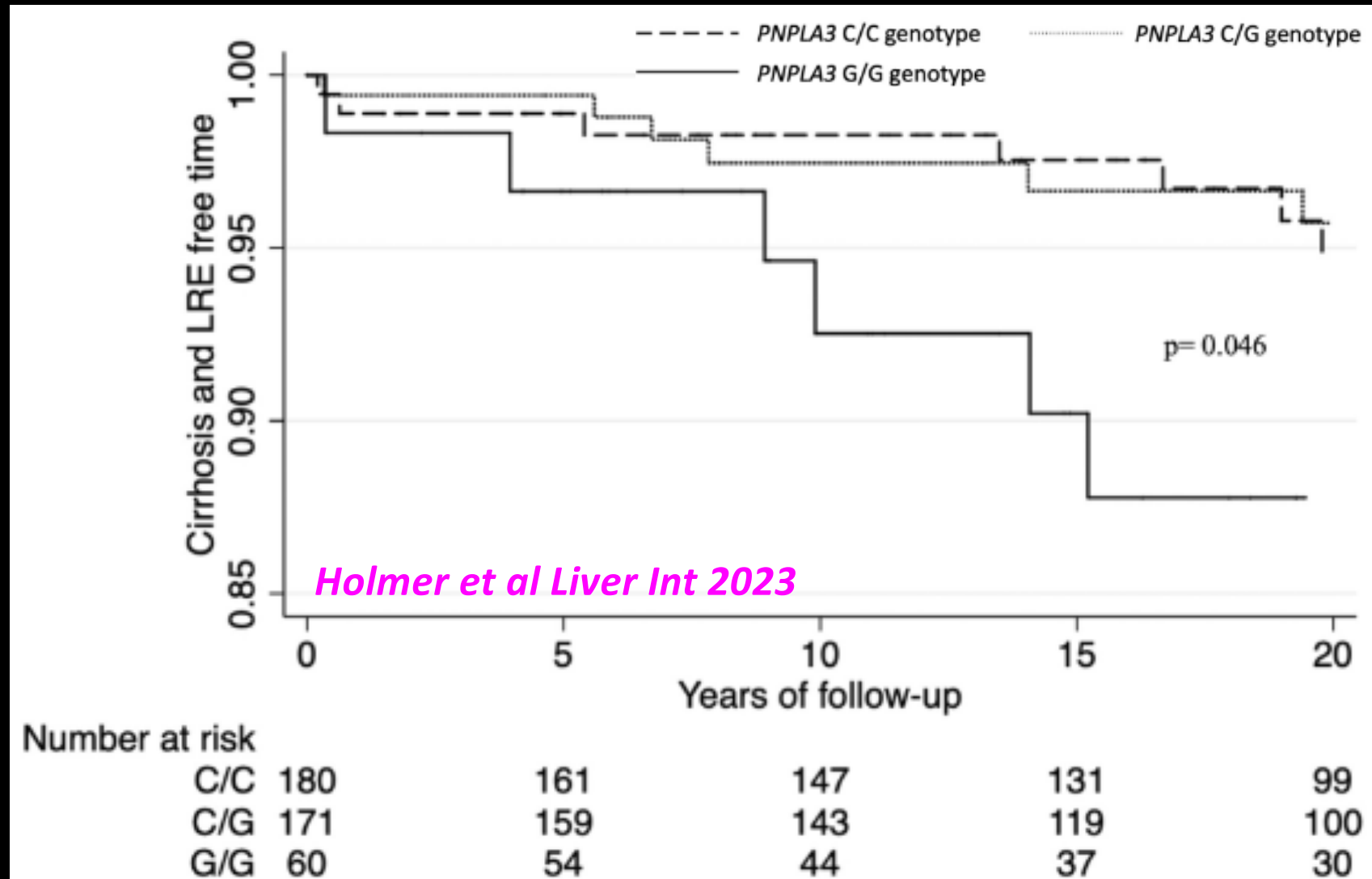
# Clinical trials on PNPLA3 downregulation

**Table 1.** Oligonucleotide-based NASH therapies in clinical development

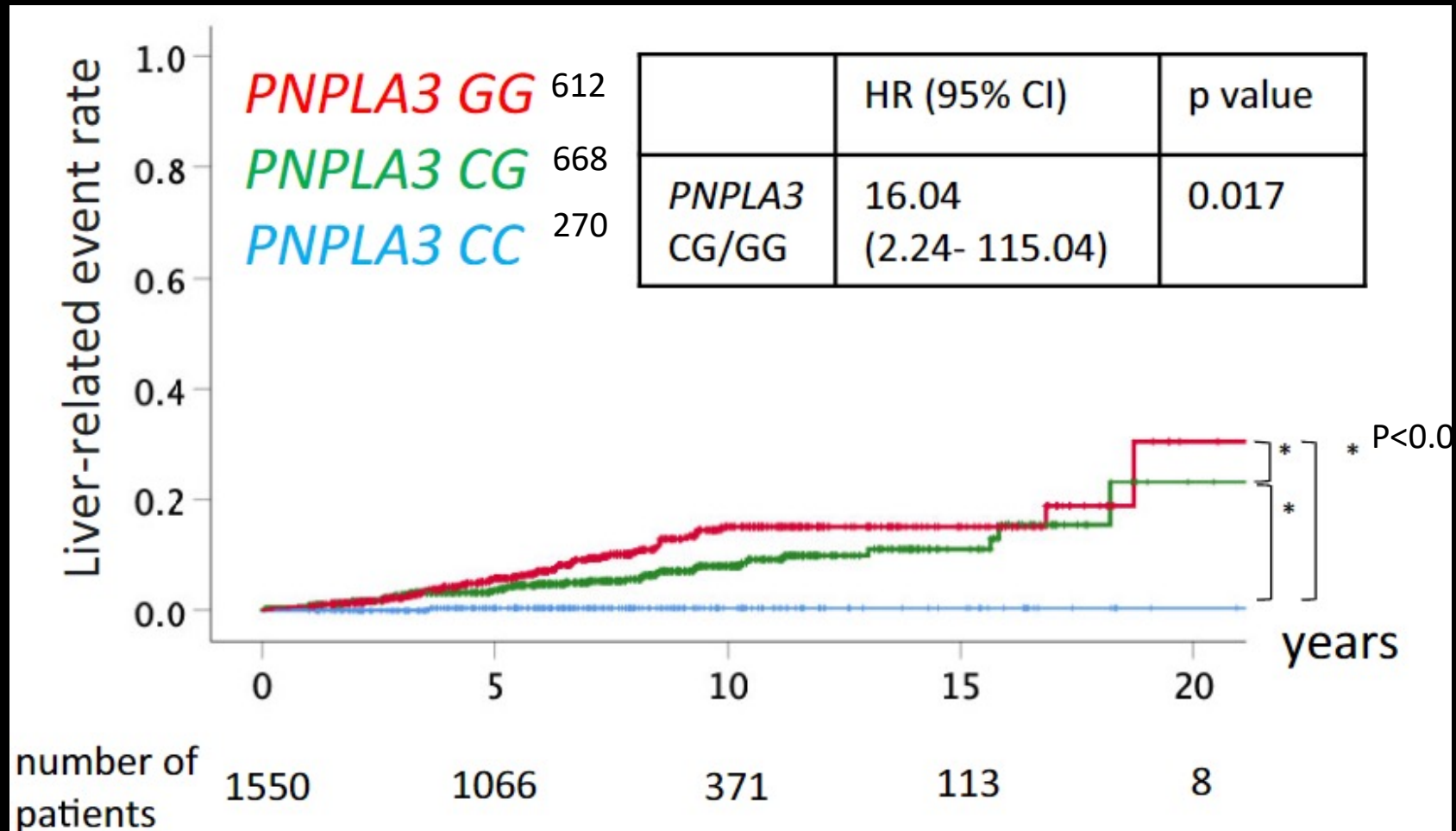
Target	Drug name (Company)	Modality	Clinical stage	Clinical trial IDs
PNPLA3	AZD2693 (AstraZeneca/IONIS)	GalNAc-ASO	Phase II	NCT05107336 <sup>d</sup> , NCT04142424 <sup>a</sup> , NCT04483947 <sup>b</sup> ,
	ARO-PNPLA3/JNJ-75220795 (Arrowhead/Janssen)	GalNAc-siRNA	Phase I	NCT04844450 <sup>b</sup> , NCT05039710 <sup>b</sup>
	LY3849891 (Eli Lilly/Dicerna)	GalNAc-siRNA	Phase I	NCT05395481 <sup>b</sup>
	AMG 609 (Amgen)	GalNAc-siRNA	Phase I	NCT04857606 <sup>d</sup>
	ALN-PNP	GalNAc-siRNA	Phase I	NCT05648214 <sup>b</sup>

**Targeting I148M Hetero- or M148M homozygotes?**

# Risk conferred by PNPLA3 variant on MASLD related cirrhosis in Swedes



# Risk conferred by PNPLA3 variant on liver related events in Japan



Tanaka et al Liver Intern 2023

**Targeting I148M Hetero- or M148M homozygotes?**



## Summary

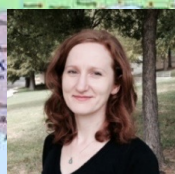
- PNPLA3 148M reduces lipid droplet remodelling in hepatocyte and hepatic stellate cells
- Downregulating PNPLA3 M148M protein is efficacious in treating MASLD *in mice*

# Acknowledgment





**Daniel Lindén**



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**Luca Valenti**



**Salvatore Petta**  
**Stefania Grimaudo**



# Bannf Canada, March 3-6 2024

## KEYSTONE SYMPOSIA

on Molecular and Cellular Biology

### **NASH and Fibrosis: From Molecular Phenotypes to Precision Therapeutics (C1)**

**March 3-6, 2024 • Fairmont Banff Springs • Banff, AB, Canada**

**Scientific Organizers: Stefano Romeo, Michael Charlton, Silvia Sookoian and Gregory Tesz**

*Supported by the Directors' Fund*

*Scholarship Deadline: November 28, 2023 / Abstract Deadline: November 28, 2023 / Discounted Registration Deadline: January 4, 2024*

**Thank you!**