ProQR®

# INVESTOR & ANALYST EVENT

December 11, 2024

# Agenda

**1. Welcome & Agenda**
Sarah Kiely

**2. Strategy overview**
Daniel A. de Boer

**3. Axiomer Platform**
Peter Beal, PhD

**4. AX-0810 for Cholestatic Diseases**
Prof. Gideon Hirschfield, MA, MB Bchir, FRCP, PhD
Gerard Platenburg

**5. AX-2402 for Rett Syndrome**
Monica Coenraads, MBA
Gerard Platenburg

**6. AX-1412 for CVD**
Gerard Platenburg

**7. AX-2911 for MASH**
Gerard Platenburg

**8. Summary & Milestones**
Daniel A. de Boer

**9. Q&A**
Daniel A. de Boer
Gerard Platenburg
René Beukema

**10. Closing**
Daniel A. de Boer

## Speakers

**Sarah Kiely**
*VP Investor Relations & Corporate Affairs*

**Daniel A. de Boer**
*Founder & CEO*

**Gerard Platenburg**
*Chief Scientific Officer*

**René Beukema**
*Chief Corporate Development Officer*

**Peter Beal,** PhD
*Professor, UC Davis; ProQR Chief ADAR Scientist; SAB member*

**Monica Coenraads,** MBA
*Founder, CEO at Rett Syndrome Research Trust*

**Prof. Gideon Hirschfield,** MA (Oxon) MB BChir (Cantab) FRCP PhD
*Professor of Gastroenterology and Hepatology, Toronto Centre for Liver Disease*

# Forward-looking statements

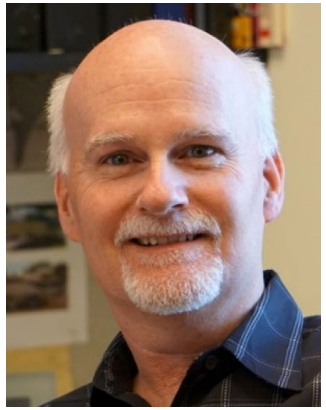
This presentation contains forward-looking statements. All statements other than statements of historical fact are forward-looking statements, which are often indicated by terms such as "anticipate," "believe," "could," "estimate," "expect," "goal," "intend," "look forward to", "may," "plan," "potential," "predict," "project," "should," "will," "would" and similar expressions. Such forward-looking statements include, but are not limited to, statements regarding our strategy and future operations, statements regarding the potential of and our plans with respect to our technologies and platforms (including Axiomer™), our preclinical model data, our pipeline targets, our other programs and business operations, our current and planned partnerships and collaborators and the intended benefits thereof, including the collaboration with Lilly and the intended benefits thereof, including the upfront payment, equity investment, and milestone and royalty payments from commercial product sales, if any, from the products covered by the collaboration, as well as the potential of our technologies and product candidates; our updated strategic plans and the intended benefits thereof, our plans to seek strategic partnerships for our ophthalmology assets, and our financial position and cash runway. Forward-looking statements are based on management's beliefs and assumptions and on information available to management only as of the date of this presentation. Our actual results could differ materially from those anticipated in these forward-looking statements for many reasons, including, without limitation, the risks, uncertainties and other factors in our filings made with the Securities and Exchange Commission, including certain sections of our annual report filed on Form 20-F. These risks and uncertainties include, among others, the cost, timing and results of preclinical studies and other development activities by us and our collaborative partners whose operations and activities may be slowed or halted due to shortage and pressure on supply and logistics on the global market; our reliance on contract manufacturers to supply materials for research and development and the risk of supply interruption from a contract manufacturer; the ability to secure, maintain and realize the intended benefits of collaborations with partners, including the collaboration with Lilly; the possible impairment of, inability to obtain, and costs to obtain intellectual property rights; possible safety or efficacy concerns that could emerge as new data are generated in research and development; general business, operational, financial and accounting risks; and risks related to litigation and disputes with third parties. Given these risks, uncertainties and other factors, you should not place undue reliance on these forward-looking statements, and we assume no obligation to update these forward-looking statements, even if new information becomes available in the future, except as required by law.

# Strategic Overview

*Presenter: Daniel A. de Boer*

# Peter Beal, PhD

*ProQR Chief ADAR Scientist & SAB member, Professor UC Davis*



- Professor in the Department of Chemistry at the University of California at Davis and Director of the NIH-funded UC Davis Chemical Biology Graduate Program
- Advanced understanding of the structures and mechanism of action for the ADAR enzymes responsible for adenosine to inosine RNA editing in humans
- Led in the development of structure-guided methods for optimizing chemically modified oligonucleotides for recruitment of RNA-binding proteins including ADARs
- Teaches organic chemistry at the undergraduate level and several classes in nucleic acids chemistry and chemical biology at the graduate level
- Over 100 peer-reviewed publications in the field of RNA chemical biology and mentored over 50 Ph.D. and M.S. degree students
- ProQR Chief ADAR Scientist, Scientific Advisory Board

# Axiomer™ advancing to value inflection

**Innovative ADAR-enabled RNA editing science driving advancement of Axiomer**

*supported by robust IP estate*

**High impact strategic partnerships**

*Eli Lilly, Rett Syndrome Research Trust*

**Pipeline with transformative potential for diseases with high unmet medical needs**

*work at root cause*

**Experienced team driving execution**

**Runway into mid 2027**

*€89.4 million cash and cash equivalents as of end of Q3, plus $82.1 million gross proceeds from October financing providing runway into mid-2027*


# Axiomer™ Platform

*Driving innovation in the ADAR RNA editing field*

Presenter: Peter Beal, PhD

# Axiomer™ RNA-editing platform technology

## Versatile

- Ability to target multiple organs and a wide range of diseases with numerous applications
- Potential to include protective variants
- Designed to target a variety of RNA species (mRNA, miRNA, lncRNA)

## Safety

- No permanent changes
- No irreversible DNA damages and less risk of permanent side effects

## High specificity

- Highly targeted therapeutic with potential to minimize off-target effects and reduce the risk of adverse reactions

## Transient

- Provide a long-lasting therapeutic effect that does not require frequent dosing
- Potential to target diseases for which permanent changes would be deleterious

## No viral vector

- No risk of immunogenicity or capacity limitation due to the vector
- Efficient development and faster production increase the chance to reach market

## Endogenous ADARs

- Leverage body's potential to treat disease
- Less risk of off-target effect vs. exogenous ADARs

ADAR: Adenosine deaminase acting on RNA, mRNA: messenger RNA, miRNA: microRNA, lcRNA: long non-coding RNA

# ProQR's Axiomer™ ADAR journey since 2014

ProQR invents oligo mediated RNA Editing recruiting endogenous ADAR

**2014**

Key ADAR patents get granted in EU and US

**2020-2023**

ProQR pivots to solely focus on ADAR editing

**2022**

ProQR's ADAR patents win opposition cases filed by strawmen across the world

**2023-2024**

**2014-2018+**

ProQR files key patents that protect ADAR mediated RNA editing broadly

**2015-2021**

ProQR optimizes the ADAR platform in stealth

**2021**

ProQR and Eli Lilly enter into first 5 target partnership worth $1.25B

**2022**

ProQR and Eli Lilly expand partnership to 10 targets worth ~$3.9B

**2023**

ProQR demonstrates >50% editing in CNS and liver in NHP and announces pipeline

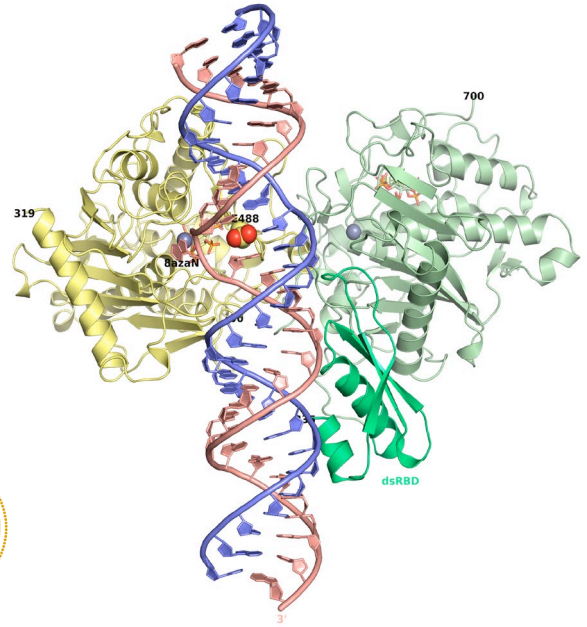
**2024**

- ProQR first in the field to report a disease relevant biomarker effect using Axiomer in NHP. Initial indication of good safety profile.
- Initial clinical validation of ADAR editing

ADARs: Adenosine deaminases acting on RNA, EONs: Editing oligonucleotides

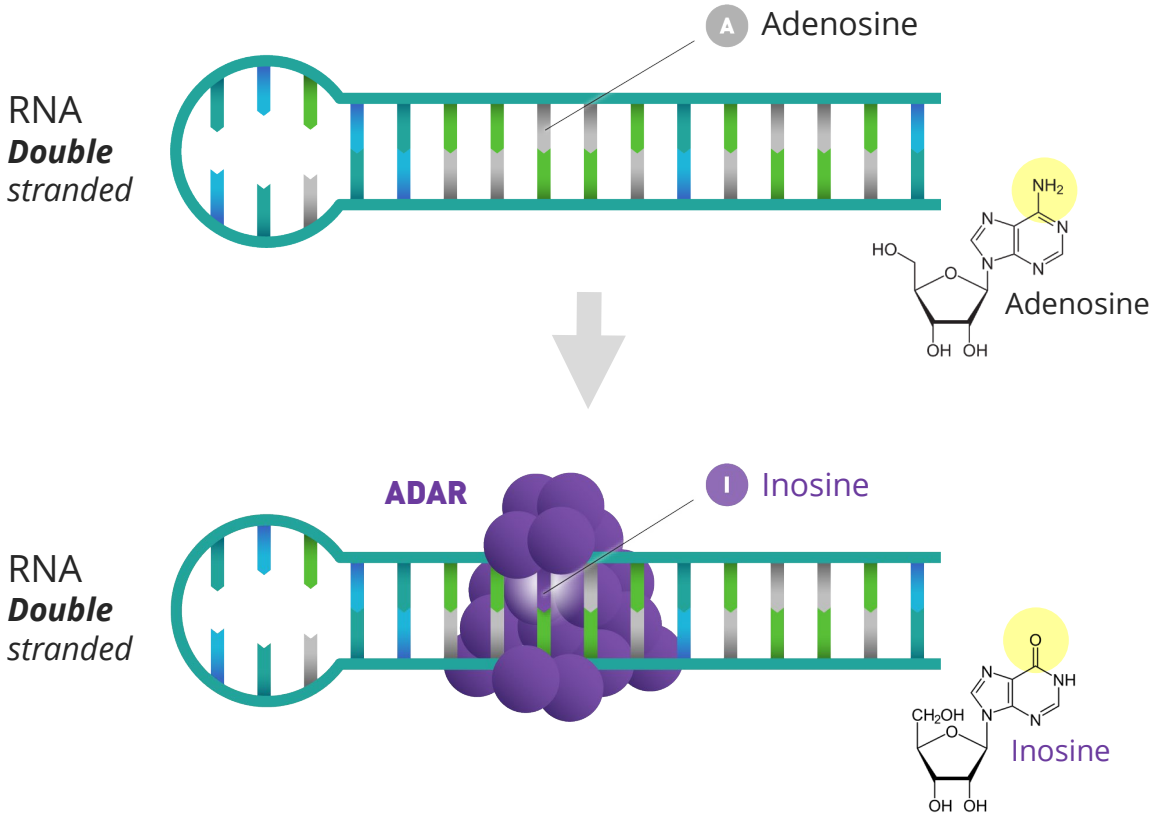
# What is ADAR editing?

**ADAR** *(Adenosine Deaminase Acting on RNA)*




Enzyme that performs specific form of natural RNA editing, called **A-to-I editing.** During A-to-I editing an **A nucleotide (adenosine)** is changed into an **I nucleotide (inosine)**

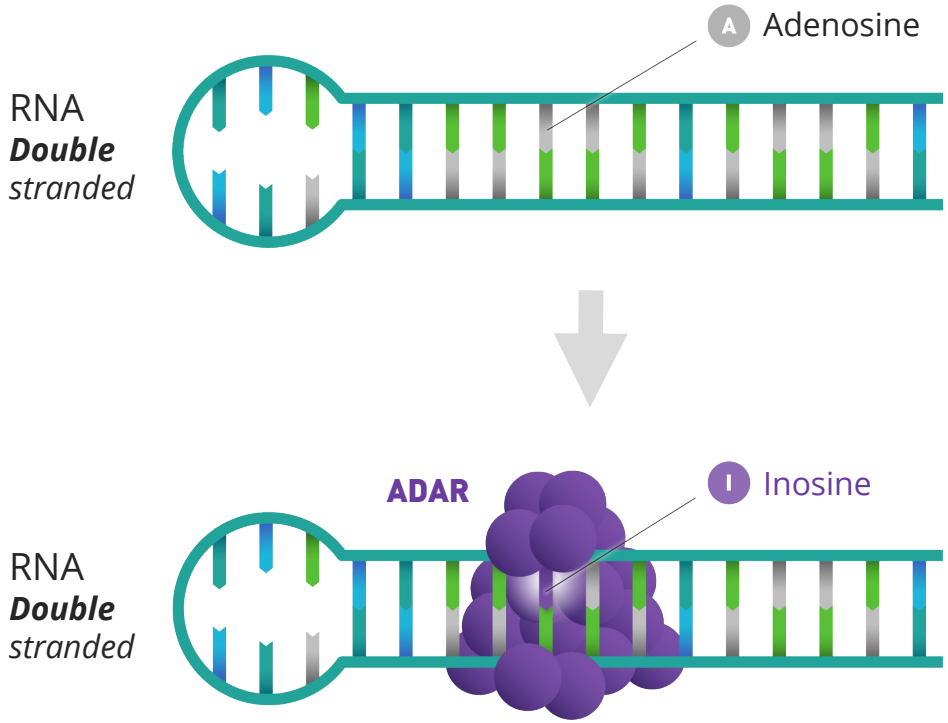
**Natural ADAR editing** *(A-to-I)*



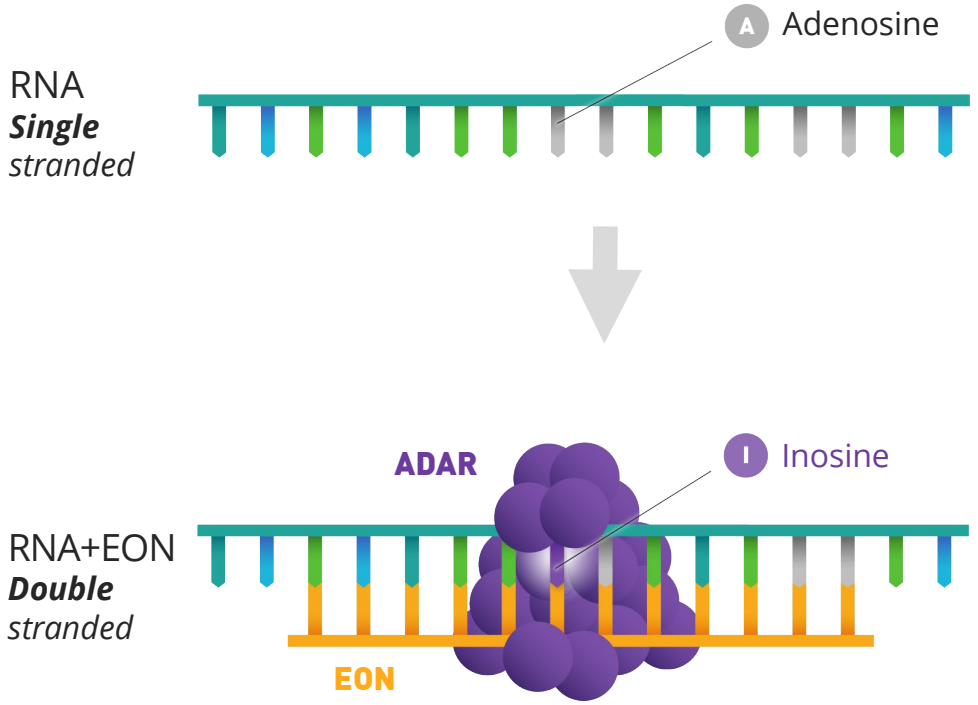
# Axiomer™ EONs unlock cellular machinery potential to treat diseases

*By attracting ADARs and allowing highly specific editing*

**Natural ADAR editing** *(A-to-I)*

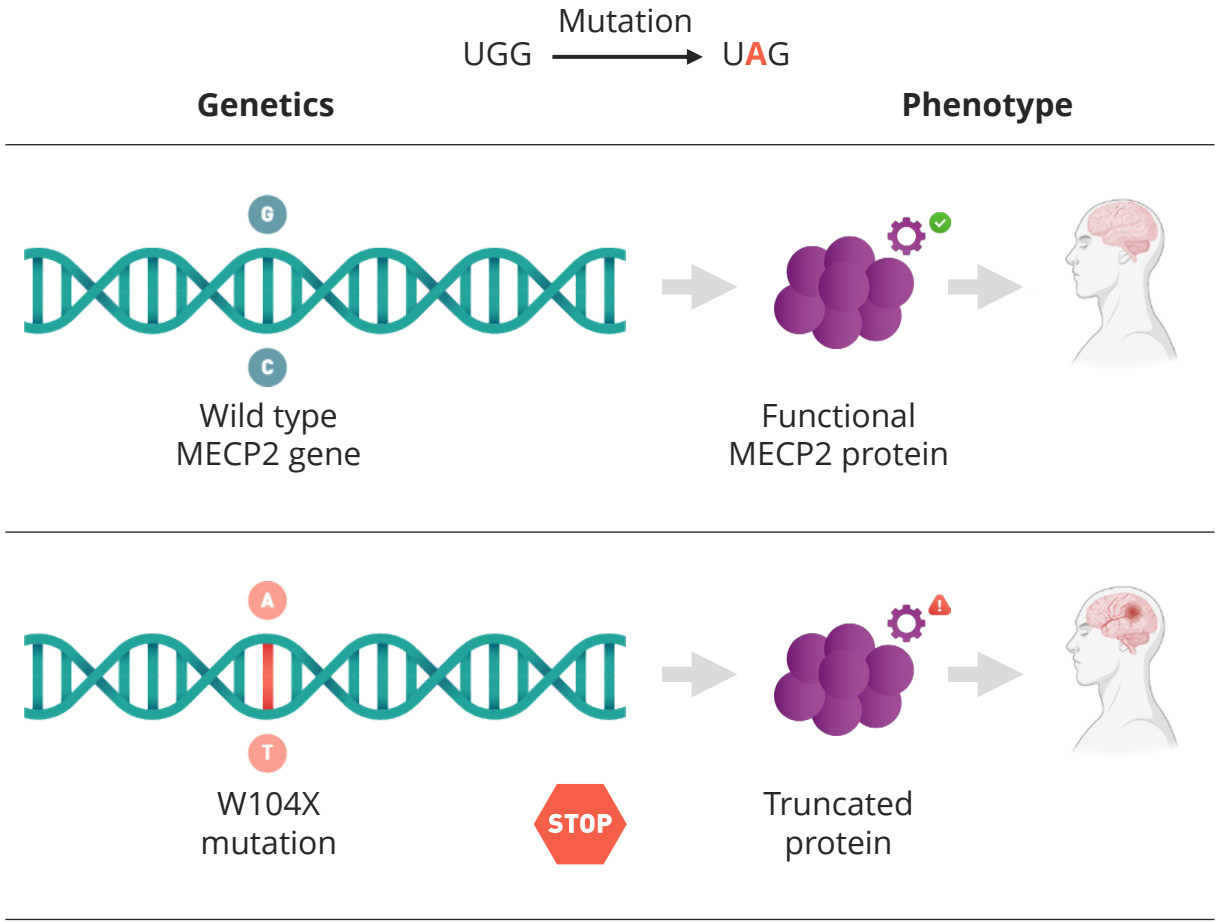


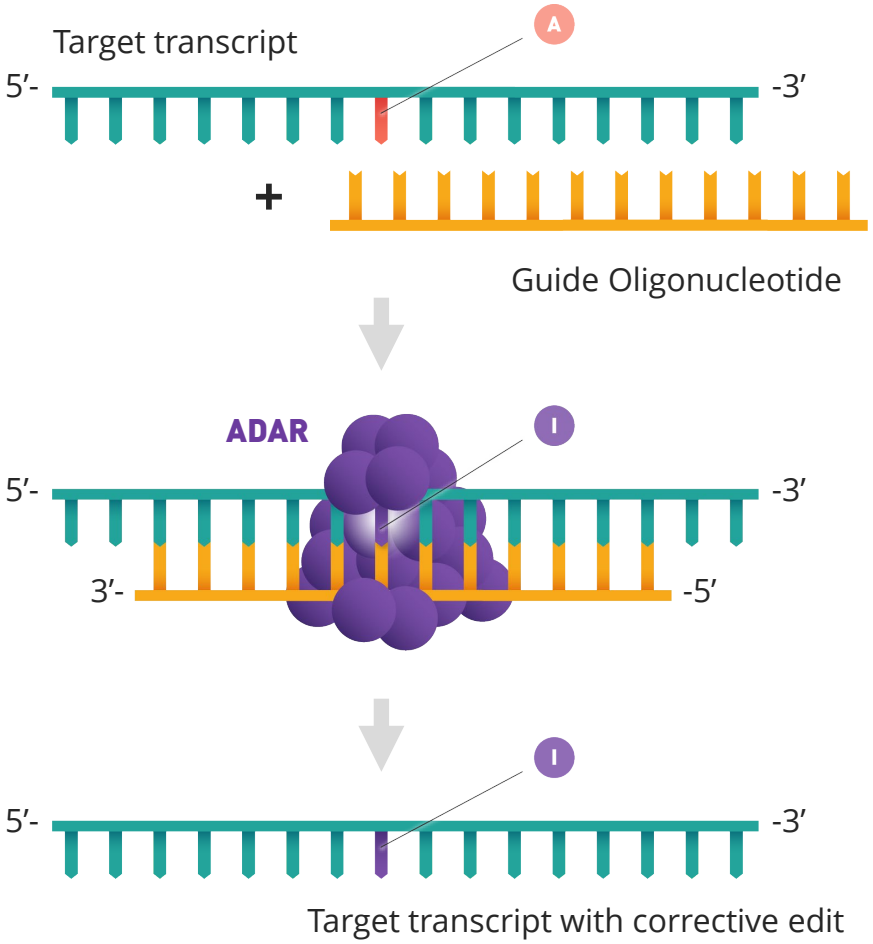

**Editing Oligonucleotide (EON)-directed therapeutic editing** *(A-to-I)*




ADARs: Adenosine deaminases acting on RNA.

# Oligonucleotide-directed RNA editing



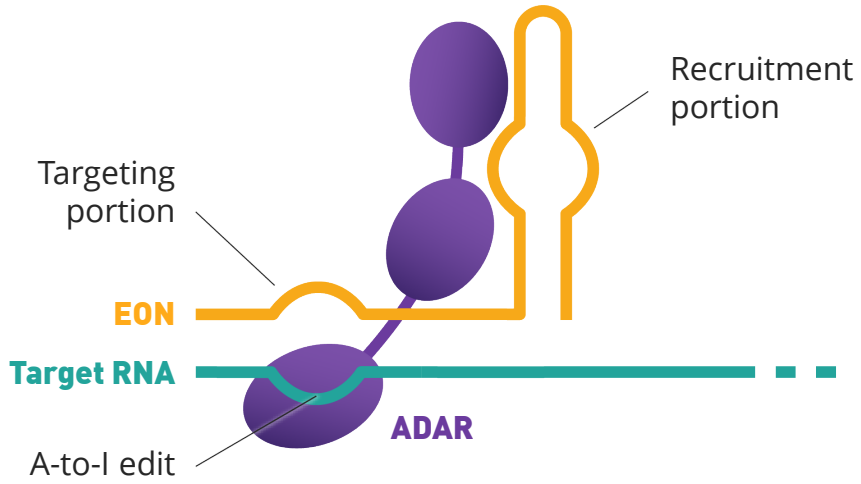




Reference: Doherty EE, Beal PA. Mol Ther. 2022 Jun 1;30(6):2117-2119.

# Driving innovation in the RNA field with Axiomer™ editing oligonucleotides

## 1st Axiomer EONs generation

*relate to (chemically modified) oligonucleotides that comprise*

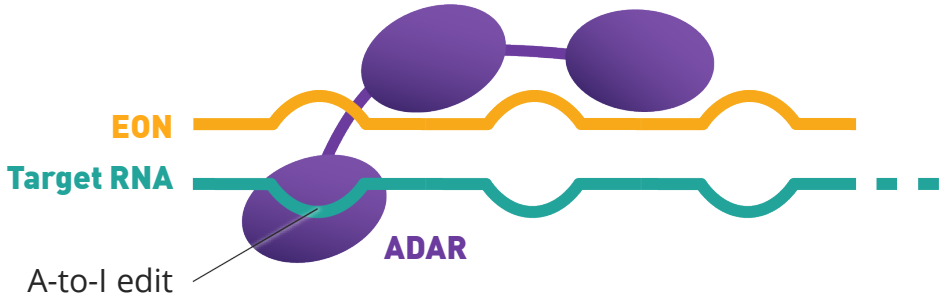



- **A targeting portion** for binding to a target RNA incl. target adenosine
- **A recruitment portion** (hairpin structure) for recruiting **endogenous** ADAR to edit the target adenosine

Patents: Granted appeal pending EP 3 234 134 B1; Granted US 10,676,737; Granted US 11,781,134

## 2nd Axiomer EONs generation

*relate to oligonucleotides that comprise*



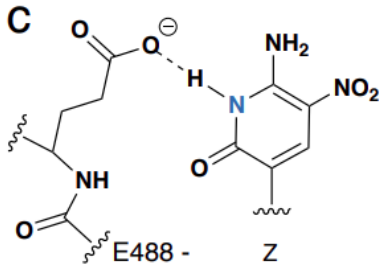

- **No hairpin structure**
- One or more wobbles and/or mismatches, and chemical modifications in the base, ribose sugar and/or linkage to **increase activity as well as stability** and are still able to recruit **endogenous** ADAR to edit the target adenosine**.**

Patents: Granted US 10,941,402; Granted US 11,851,656; Allowed US 18/296,912

# ProQR leading research to optimize editing oligonucleotides for therapeutic use

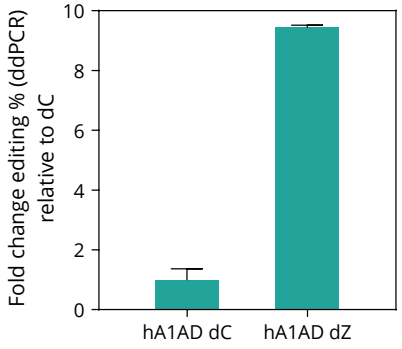
## Modification of the orphan base

*dZ in EER to increase ADAR activity*



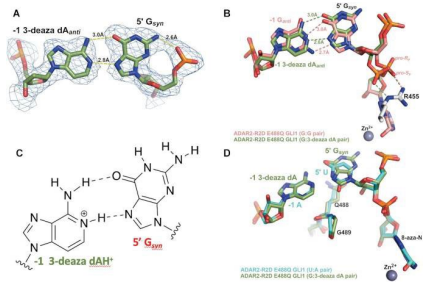

**RNA editing of *SERPINA1* E366K in A1AD patient hepatocytes**

*Transfection of 100nM EON, N=2, 48 hours*



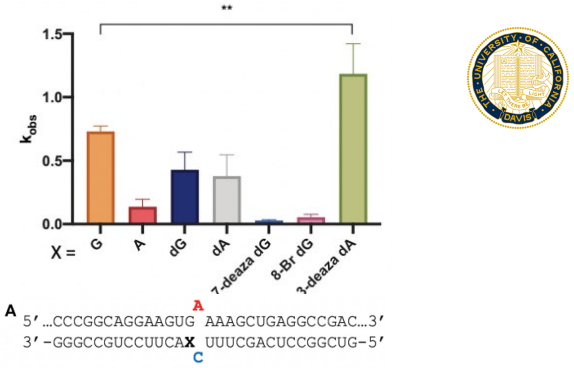

## Modification of the base opposite to 5'g

*3-deaza-dA in EER to increase editing activity in 5'G unfavorable context*




***In vitro* deamination kinetics for ADAR2 and duplex RNAs derived from *hMECP2* R255X**

*100nM ADAR2, 3 technical replicates, mean, SD*

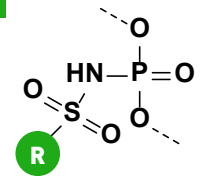



## Linkage modifications in the ABR

*PN and PMe linkages in the ABR to increase stability, EON liver concentration and target engagement*

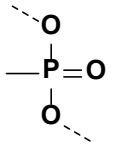
**Phosphoroamidate linkage**

PN




**Methylphosphonate linkage**

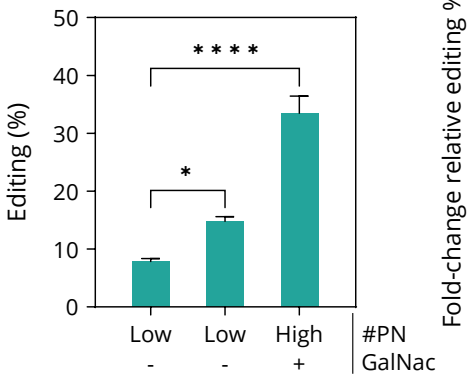
PMe




R = Me: Mesyl-PN

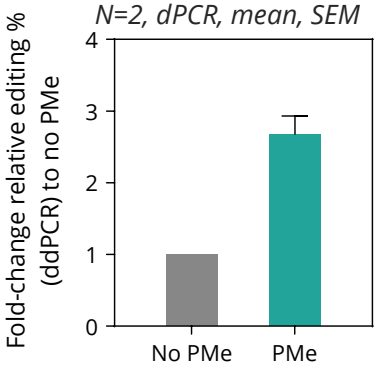
**RNA editing of *ActB* in liver**

*C57Bl/6J mice, 7d, 3x10mg/kg, SC, N=4, dPCR, mean, SEM*




**RNA editing of *APP* in HepG2 cells**

*Gymnosis, 5d, 5μM single dose, N=2, dPCR, mean, SEM*



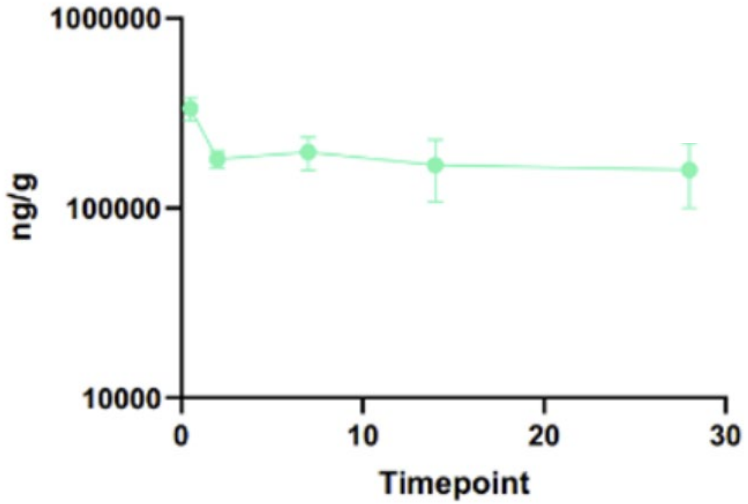

Adapted from Doherty EE, et al. Nucleic Acids Res. 2022;50(19):10857-10868. Statistical significance between groups was determined using one-way ANOVA with Tukey's multiple comparisons test or an unpaired t-test with Welch's correction; **P < 0.01; ***P < 0.001; ****P < 0.0001.

# Sequence optimization enables stable editing oligonucleotides with prolonged PK

*Learnings from advanced programs inform editing optimization*

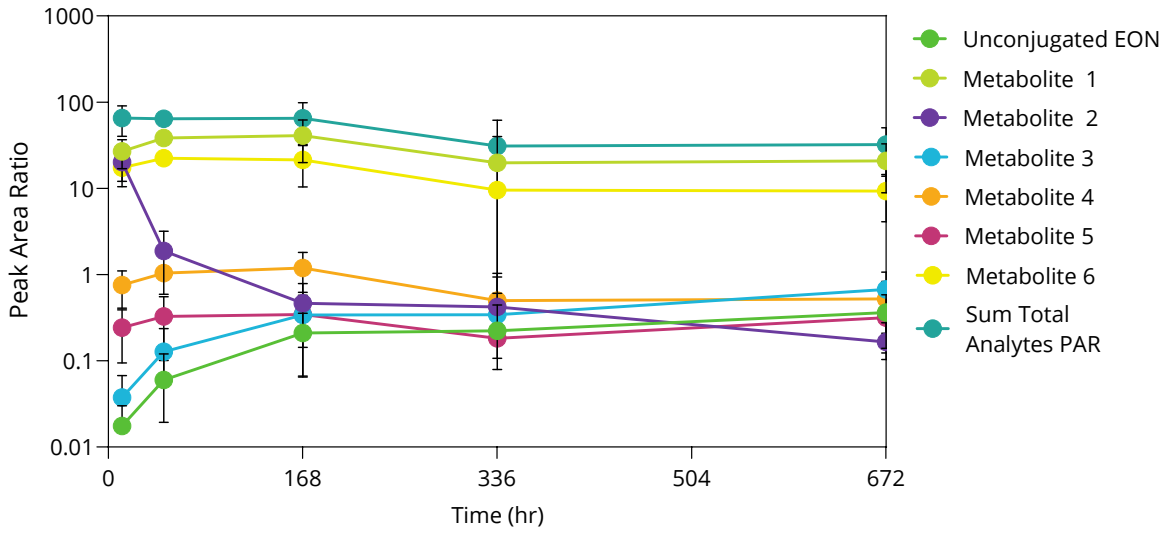
**EON11 concentration in liver of mice disease model**

*Hybridization-HPLC, n=6, 30mg/kg, EON11, SC, GalNAc conjugation, up to 4 weeks*




**EON11 metabolites in liver of mice disease model**

*LC-MS, n=6, 30mg/kg, EON11, SC, GalNAc conjugation, up to 4 weeks*

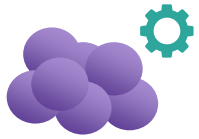
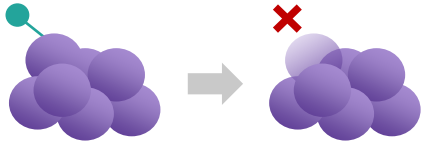



- Rapid absorption in the liver and long half-life of EON11 in liver measured – around 80 days
- EON show high stability with no metabolites observed for oligonucleotide itself

- Up to six metabolite were identified and all were the metabolite of the GalNAc entity
  - Most represented is linker between EON and GalNAc moiety
  - Others were a combination of different cleavages of different GalNAc arms or within the linker

Perkins E. 726. Complex Metabolism and Prolonged PK/PD of a GalNAc-Conjugated Editing Oligonucleotide (EON) in Mice. ASGCT 27th Annual Meeting Abstracts; *Molecular Therapy,* Volume 32, Issue 4, 1 - 889

# Creating a new class of medicines with broad therapeutic potential

| Correction | Protein modulation | | |
|---|---|---|---|
|  |  |  |  |
| **Mutations correction**<br>Thousands of G-to-A mutations, many of them described in literature | **Alter protein function or include protective variants**<br>Modified proteins achieving loss- or gain-of-functions that help addressing or preventing diseases | **Disrupt >400 different types of PTMs**<br>Regulate protein activity, change localization, folding, preventing immune escape or slowing down degradation | **Change protein interactions**<br>Changes localization, folding, protein function or prevents immune escape of glycosylated tumor antigens |
| Mutation correction leading to protein recovery | Variant resulting in a dominant negative effect | Reduction of protein phosphorylation altering protein function | Variant impacting protein interaction with sugar |

# Axiomer™ RNA editing science translating toward therapeutic applications

## Science

- Harnessing advanced knowledge of ADAR and oligonucleotide science
- Pioneering the optimization of editing oligonucleotides (EONs) to achieve best-in-class therapeutic solutions

## Versatile applicability

- Demonstrating proven success in correcting genetic mutations and enabling diverse protein modulation strategies
- Platform with potential to address diverse conditions rooted in human genetics

## Leadership position

- Driving innovation in the ADAR RNA editing science with Axiomer EONs since 2014
- Dominant IP position to drive ADAR-mediated RNA editing platform innovation

# AX-0810 Program

*Targeting NTCP to address cholestatic diseases unmet medical need at the root cause*

Presenters: Prof. Gideon Hirschfield, Gerard Platenburg

# AX-0810 RNA editing therapy targeting NTCP for cholestatic diseases

Cholestatic diseases have high unmet medical need. Patients accumulate bile acids in liver leading to fibrosis and ultimately liver failure.

Initial indications are **Primary Sclerosing Cholangitis** affecting adults and Congenital **Biliary Atresia** affecting pediatrics early in life. Both conditions have no approved therapies and may require liver transplantation.[1,2]

- **Biliary Atresia** is projected to affect ~20,000 pediatric individuals in US and EU.
- **Primary Sclerosing Cholangitis** is projected to affect more than 80,000 individuals in US and EU.

AX-0810 is a unique therapeutic approach leading to a potentially disease modifying therapy by targeting the NTCP channel which is responsible for majority of bile acid re-uptake in liver cells.


[1]Trivedi PJ, et al. Clin Gastroenterol Hepatol. 2022 Aug;20(8):1687-1700.e4; [2]Schreiber RA, et al. J Clin Med. 2022 Feb 14;11(4):999

# Prof. Gideon Hirschfield MA (Oxon), MB BChir (Cantab), PhD, FRCP

*Professor of Gastroenterology and Hepatology, Toronto, Ontario, Canada*

- Lily and Terry Horner Chair in Autoimmune Liver Disease Research
- Director, The Autoimmune and Rare Liver Disease Programme, Toronto General Hospital
- Professor, Division of Gastroenterology and Hepatology, University of Toronto
- Prof. Gideon M. Hirschfield is an experienced and highly focused clinician-scientist specialising in autoimmune and cholestatic liver diseases. He holds the Lily and Terry Horner Chair in Autoimmune Liver Disease Research at the Toronto Centre for Liver Disease, Toronto General Hospital, and serves as a Professor of Medicine in the Division of Gastroenterology and Hepatology at the University of Toronto.
- Prof. Hirschfield completed undergraduate studies in Medicine from the Universities of Oxford and Cambridge and subsequently was awarded a PhD from the University of London in 2006. He completed specialist training in Internal Medicine, Gastroenterology and Hepatology in London, Cambridge and Toronto.
- An internationally recognised expert, Prof. Hirschfield has published over 350 peer-reviewed articles, including lead authorship in high-impact journals such as the New England Journal of Medicine, The Lancet, and Nature Genetics.
- His research focuses on advancing therapies for autoimmune and cholestatic liver diseases with the clear goal of preventing the need for transplantation alongside improving patient quality of life

# State of the art in cholestatic liver disease

*Gideon Hirschfield*

*Toronto, Canada*

**THE AUTOIMMUNE & RARE LIVER DISEASE PROGRAMME**

PBC, PSC, AIH, Hepato-biliary IgG4-RD & Genetic Cholestasis

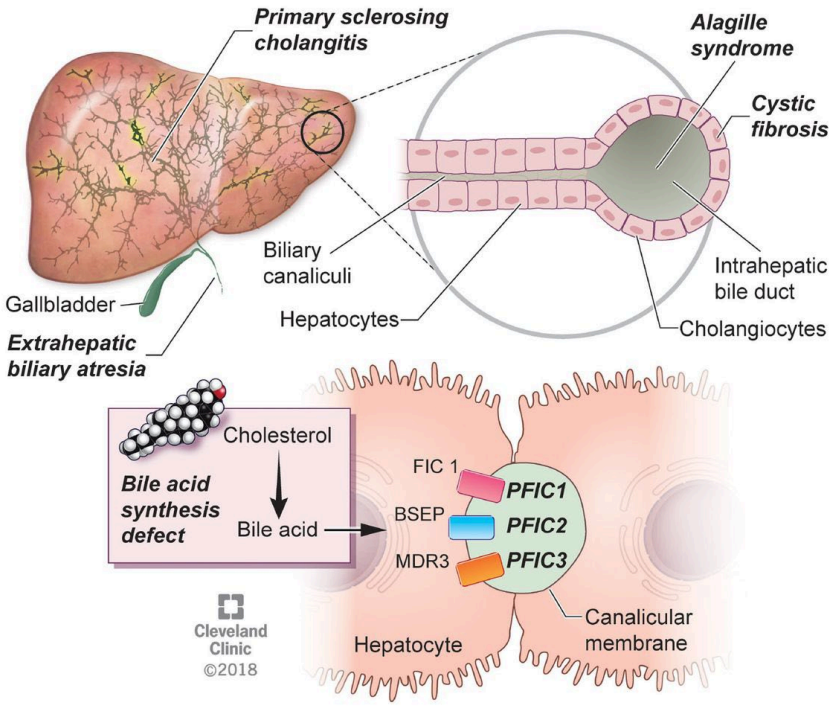
**Care** **Teaching** **Research**

# Disclosures

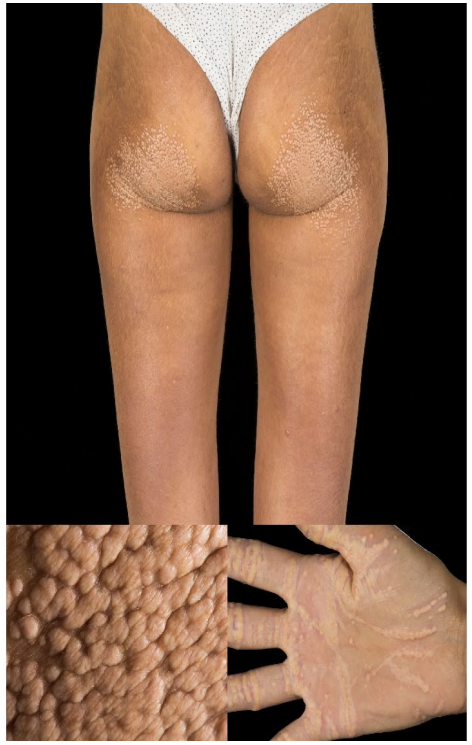
- Intercept Pharmaceuticals, Inc./Advanz
- Ipsen
- CymaBay Therapeutics/Gilead Sciences
- Pliant Therapeutics
- Escient
- Mirum
- GSK
- Kowa
- Chemomab
- Falk
- ProQR

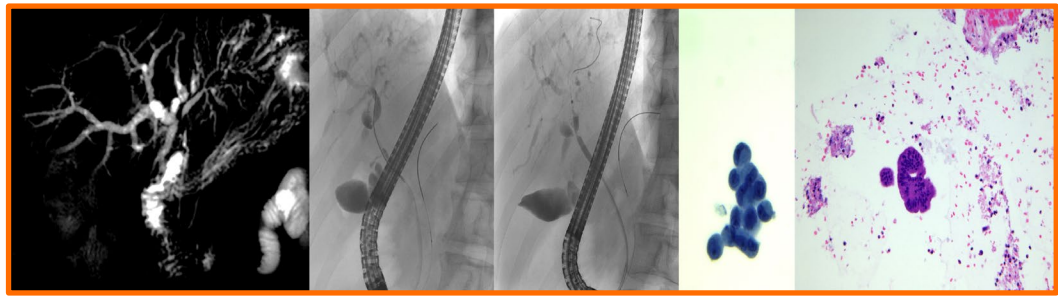
# Cholestatic liver disease

## Where unmet need in Hepatology practice remains




BSEP = bile salt export pump; FIC 1 = familial intrahepatic cholestasis protein 1; MDR3 = multidrug resistance protein 3; PFIC = progressive familial intrahepatic cholestasis



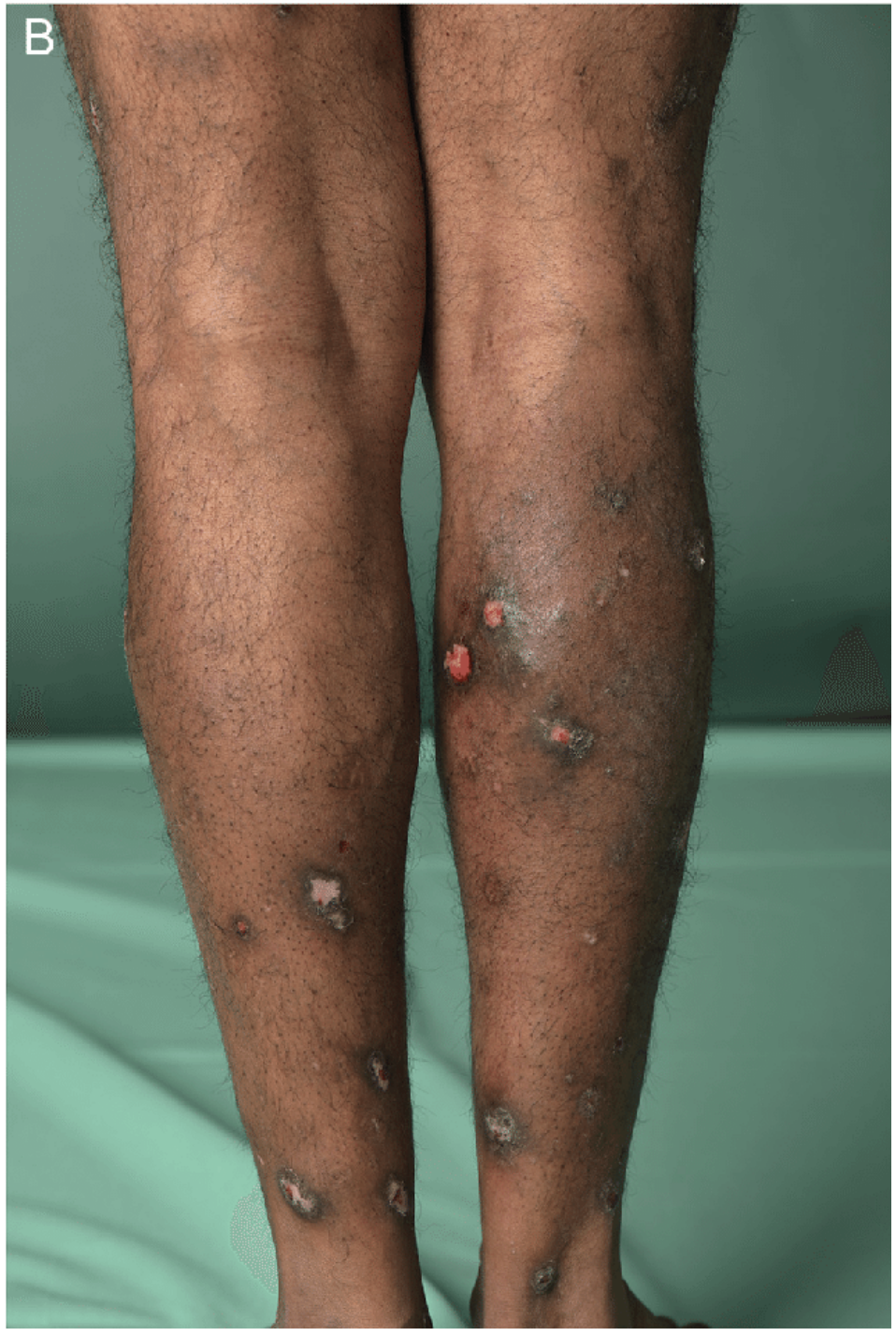


Praveen Kumar Conjeevaram Selvakumar et al. CCJM 2019;86:454-464

Paediatric/genetic cholestatic liver disease incl. biliary atresia

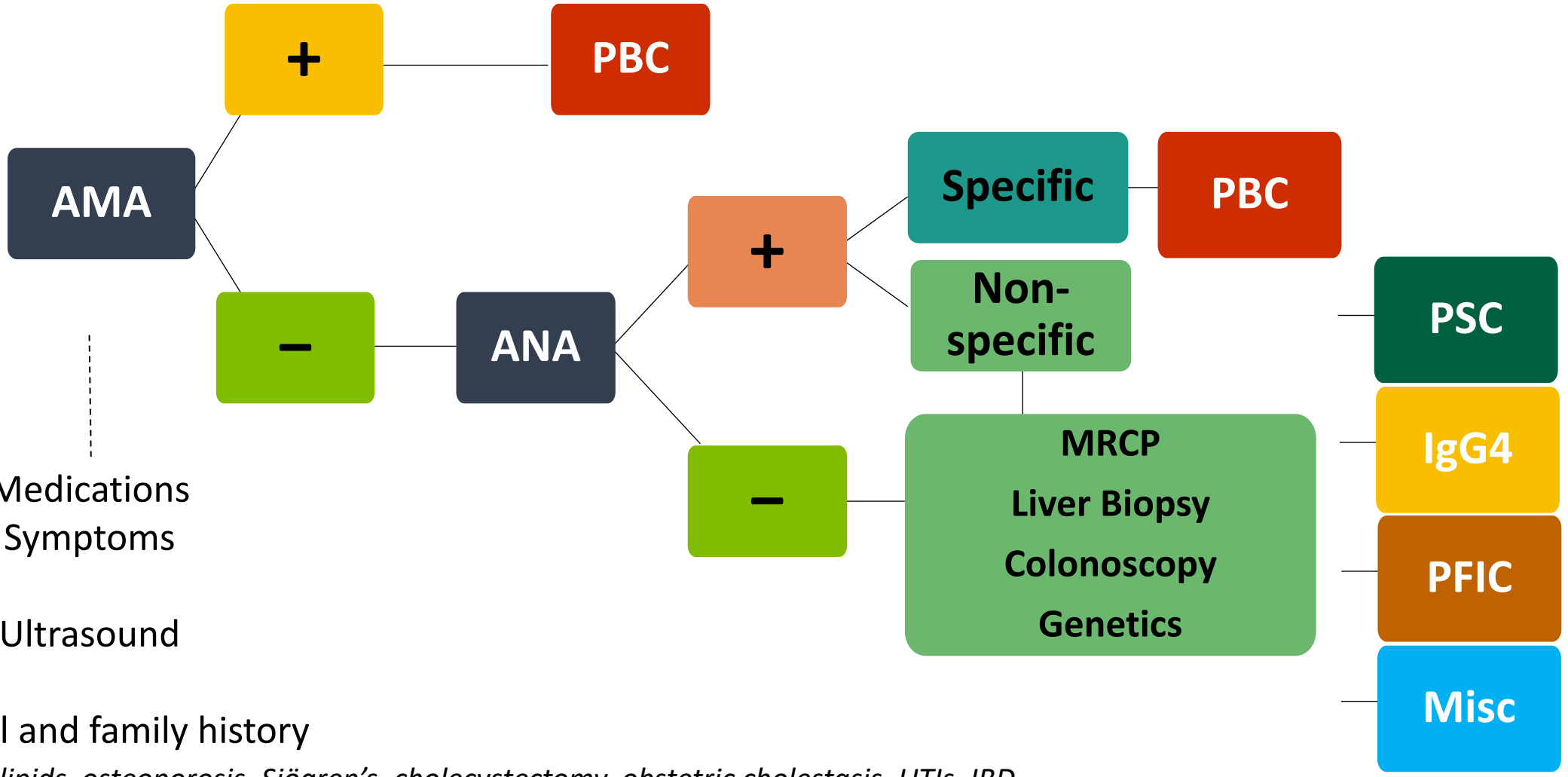
Primary biliary cholangitis

Primary sclerosing cholangitis




https://www.researchgate.net/figure/Scratch-lesions-Pruritus-of-different-origins-may-lead-to-scratch-lesions-Two-examples_fig3_308094035

# Investigating the adult patient with cholestasis




Medications
Symptoms

Ultrasound

Personal and family history

*e.g. thyroid, celiac, lipids, osteoporosis, Sjögren's, cholecystectomy, obstetric cholestasis, UTIs, IBD*

# Investigations

| | |
|---|---|
| Albumin (g/L): | **33** (L) |
| Bilirubin, Total (mg/dl): | **2**(H) |
| Bilirubin, Direct: | 1(H) |
| AST (U/L): | 82 (H) |
| ALT (U/L): | 82 (H) |
| Alkaline Phosphatase (U/L): | **330** (H) |
| GGT (U/L): | 171 (H) |

| | |
|---|---|
| Hb (g/L): | 89 (L) |
| WBC (10*9/L): | 3.9 (L) |
| Plt (10*9/L): | **104** (L) |
| MCV (fL): | 79.9 (L) |

## Liver Stiffness

Result: **17.0 kPa**
Valid measurements: 10
IQR: 2.7 kPa. IQR/Median: 16 %.

*India (May 2023):*
Grade 1 varices / cirrhosis & splenomegaly

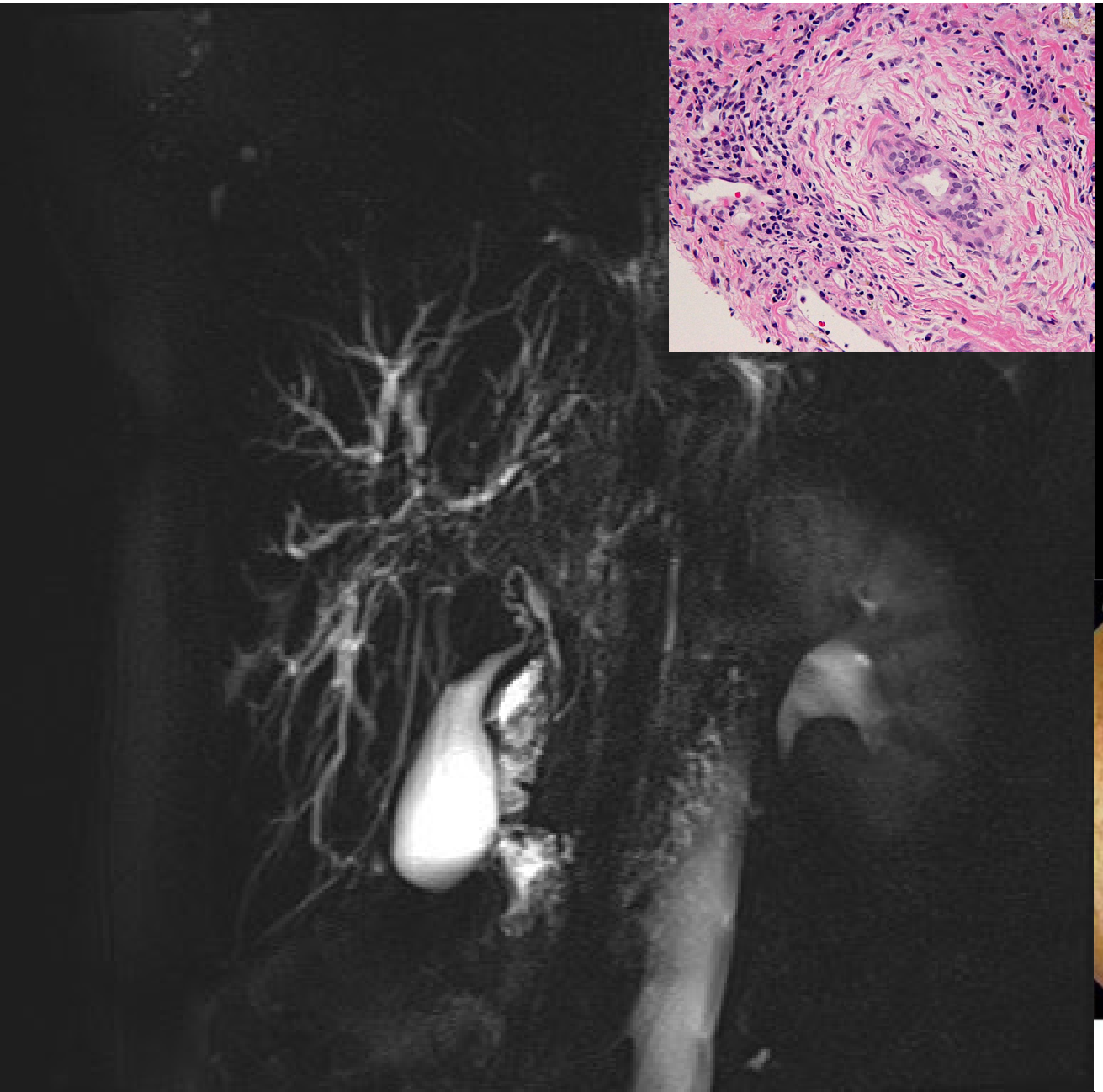
## Immunology

| | |
|---|---|
| ANA Pattern 1 | **Centromere** |
| ANA Titre 1 | **1:640 or greater** |

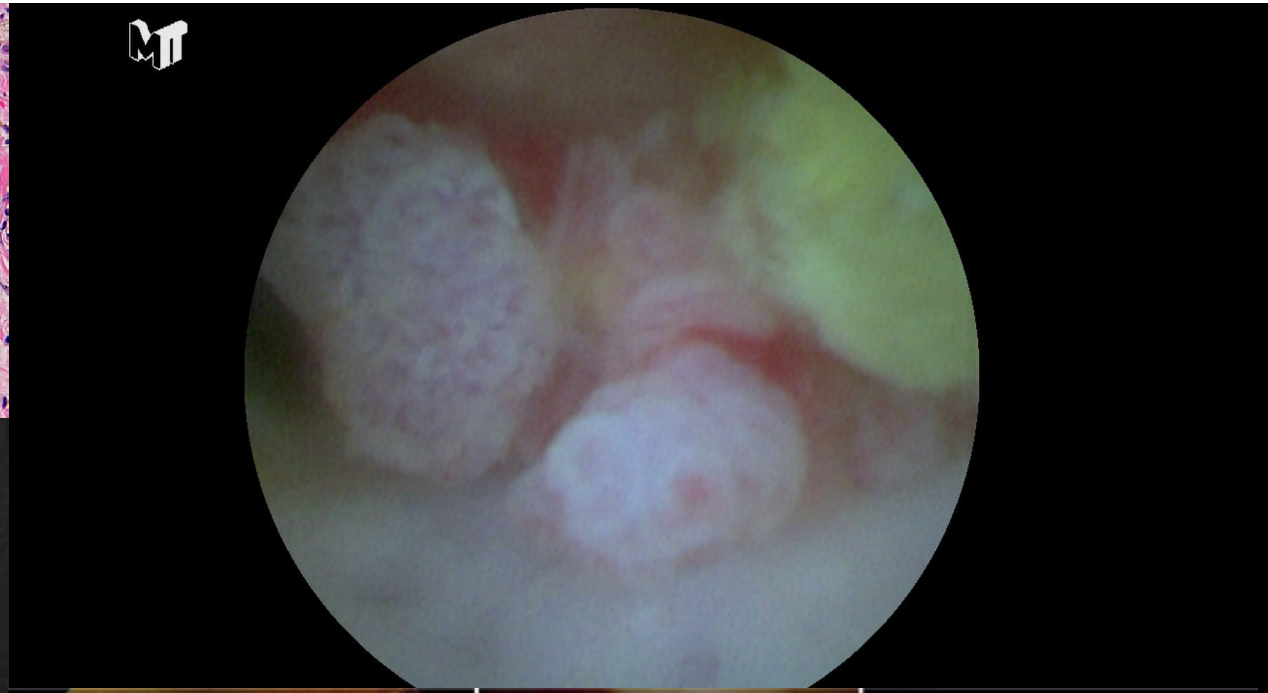
IgG 14.0 g/L
IgA 3.62 g/L
IgM **3.41** g/L

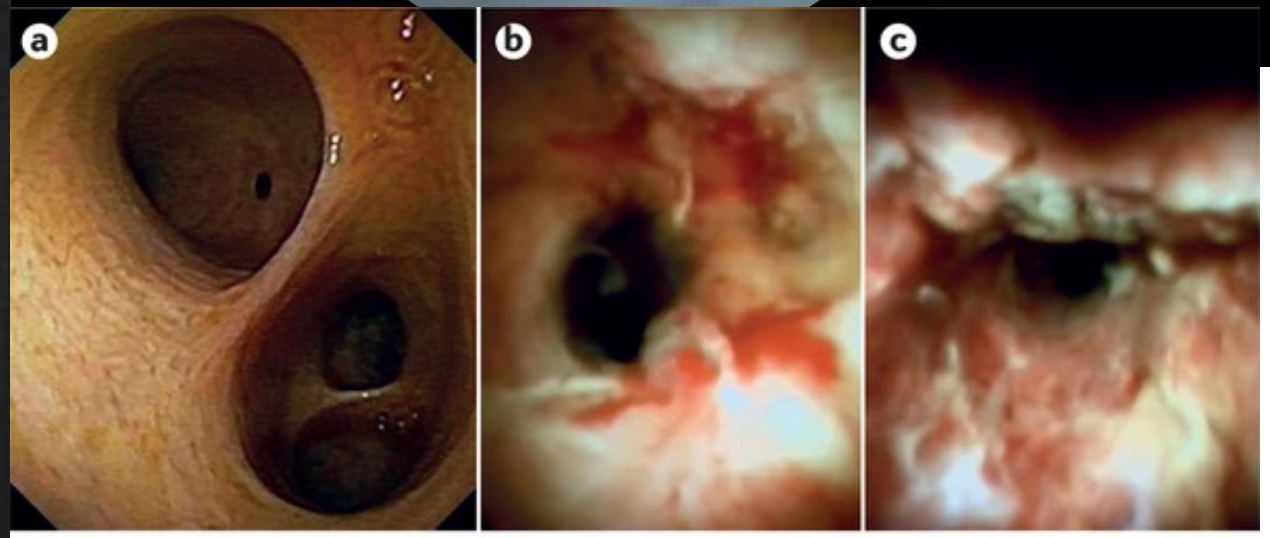
| | |
|---|---|
| **Anti AMA-M2** | **1+** |
| Anti M2-3E (BPO) | Negative |
| **Anti Sp100** | **3+** |
| Anti PML | **2+** |
| Anti gp210 | Negative |

# Living with uncertainty….









**Family...**
**Children to adult**

**Genome**

**Common genetic variation**
- HLA and non-HLA

**Rare gene disorders**
- AIRE, *GATA2, FoxP3, CTLA4*

**Exposome**

**Smoking status**
**Socioeconomic status**
**Drug exposure and xenobiotics**
**Industrial dust and solvent exposure**
**Environmental pollutants**
**Vitamin D**
**Hygiene hypothesis**

**Epigenome**

**Cell-specific methylation changes**

**Chromosomal changes**

**Histone modification**

**Microbiome**

**Reduced gut bacterial diversity**

**Increased amine producing spp.**

**Altered bile acid metabolism**

**Primary biliary cholangitis**

Predominantly women
75% above age of 50y
AMA+ / PBC-specific ANA
Elevated IgG and IgM values
Lymphocytic granulomatous cholangitis

**Autoimmune hepatitis**

Predominantly women
Any age
ANA/SMA/LKM-1/SLA-LP
Elevated IgG values
Interface hepatitis

**Primary sclerosing cholangitis**

Any age
Fibroinflammatory biliary disease
Stricturing cholangiopathy
>70% have IBD
Neoplastic predisposition

**IgG4-related cholangitis**

Predominantly older men
Stricturing cholangiopathy
Storiform fibrosis
Systemic IgG4 disease

**PSC / AIH overlap**

**Autoimmune sclerosing cholangitis ?**

**Paediatric cholestasis**

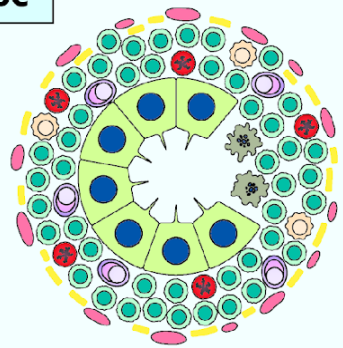
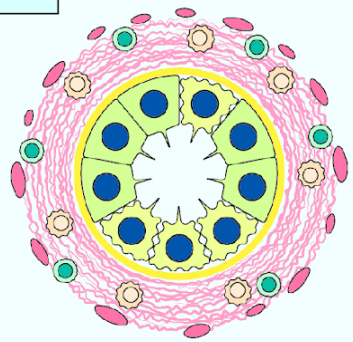
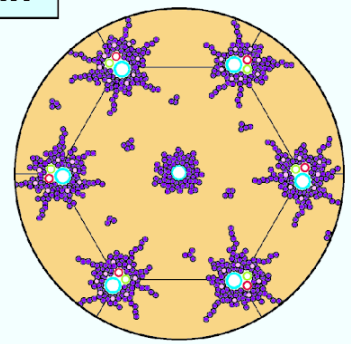
Biliary atresia
PFIC
Alagilles
Metabolic

**Symptom complex**
Pruritus, fatigue, abdominal pain, arthralgia

| | | | | |
|---|---|---|---|---|
| Incidence per 100,000: | 0.8-2.8 | 0.4-2.4 | 0.4-2.3 | ?? |
| Prevalence per 100,000: | 1.9-40.2 | 4.8-42.9 | 0.8-31.7 | ?? |

Rare but leading cause of paediatric transplant

Trivedi and Hirschfield Gut 2021

| Initiation | Pre-Clinical | Early Clinical | Advanced Clinical |
|---|---|---|---|
| **PBC**<br>  | **Histopathology:**<br>▪ Lymphoplasmacytic Portal Infiltrates<br>▪ Lymphocytic Cholangitis ± Florid Duct Lesions<br>▪ Displaced BM and PCP<br>▪ ± Granulomas<br>**Autoantibodies:**<br>▪ AMA, ANA (gp210, sp100), SMA | **Laboratory Tests:**<br>▪ Elevated ALP and ggt<br>▪ Variable elevation ALT, AST<br>▪ Elevated IgM<br>**Signs or Symptoms:**<br>▪ Cholestatic pruritus<br>▪ Fatigue<br>▪ Hyperpigmentation<br>**SOC Therapy:**<br>▪ UDCA; OCA; Fibrates | **Intolerance or Inadequate Response to UDCA:**<br>▪ Progressive Ductopenia<br>↓<br>▪ Progressive Ductular Reaction<br>↓<br>▪ Biliary Fibrosis<br>↓<br>▪ Biliary Cirrhosis |
| **PSC**<br>  | **Histopathology:**<br>▪ Focal Fibrous Obliterative Cholangitis<br>▪ Peribiliary Fibrosis<br>▪ Lymphocyte-Macrophage Portal Infiltrates<br>▪ Displaced PCP<br>**Autoantibodies:**<br>▪ pANCA (pANNA), ANA | **Laboratory Tests:**<br>▪ Elevated ALP and ggt<br>▪ ± Elevation ALT, AST<br>**Signs or Symptoms:**<br>▪ Asymptomatic<br>▪ Associated IBD<br>▪ Cholestatic pruritus<br>▪ Fatigue<br>**SOC Therapy:**<br>▪ None | ▪ Progressive Biliary Strictures<br>↓<br>▪ Fibro-Obliterative Ductopenia<br>↓<br>▪ Progressive Biliary Obstruction<br>↓<br>▪ Progressive Ductular Reaction<br>↓<br>▪ Biliary Fibrosis<br>↓<br>▪ Biliary Cirrhosis |
| **AIH**<br>  | **Histopathology:**<br>▪ Lymphoplasmacytic Portal Infiltrates<br>▪ Interface Hepatitis<br>▪ ± Central Perivenulitis<br>**Autoantibodies:**<br>▪ ANA, SMA, LKM1, SLA | **Laboratory Tests:**<br>▪ Elevated ALT, AST<br>▪ Elevated IgG<br>**Signs or Symptoms:**<br>▪ Asymptomatic<br>▪ Fatigue<br>▪ Other AI Diseases<br>**SOC Therapy:**<br>▪ 1st Line Steroids ± Thiopurine<br>▪ 2nd Line CNI or MMF | **Intolerance or Inadequate Response to 1st or 2nd Line Immunosuppression:**<br>▪ Progressive portal fibrosis<br>↓<br>▪ Bridging fibrosis<br>↓<br>▪ Post-necrotic cirrhosis |

Trivedi et al. Gastroenterology 2024

# So many questions in PSC and reasons to do better for our shared patients

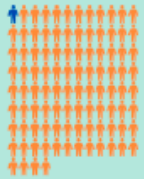
## Effective treatments will only come with understanding what causes disease

| | | |
|---|---|---|
| Why me?<br>Why children?<br>Why men more than women?<br>Why the symptoms? | Why the biliary tree?<br>Why the association with IBD?<br>Why is cancer an issue?<br>Why does PSC recur post-transplant? | Why so difficult to treat?<br>Who to study?<br>How to cross the treatment goalpost?<br>What to treat: liver or bowel? |
| Epidemiology/Genetics/Exposome | Basic science: cells, animals, human | Clinical science |

# Primary Sclerosing Cholangitis-Inflammatory Bowel Disease: Epidemiology, Mortality, and Impact of Diagnostic Sequence


Population ~15 million
26% self-identify as a visible minority

**PREVALENCE**



1 person living with PSC-IBD per 100 living with IBD

PSC-IBD: 5.5 per 100,000 PY
IBD: 588 per 100,000 PY

**INCIDENCE**

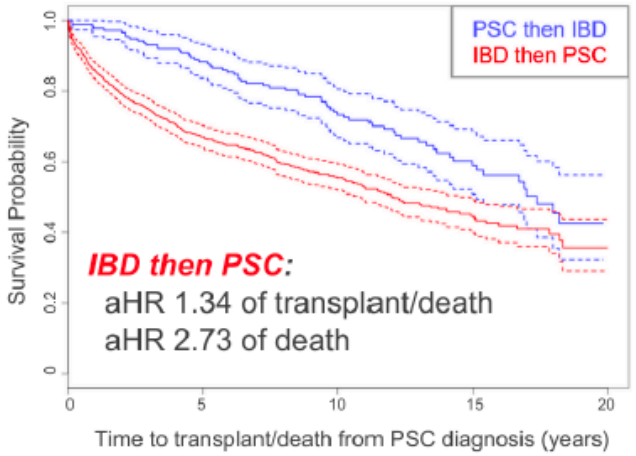
1 PSC-IBD diagnosis per 50 IBD diagnoses

PSC-IBD: 0.47 per 100,000 PY
IBD: 24.6 per 100,000 PY

**PSC-IBD COHORT CHARACTERISTICS**

Higher socioeconomic status

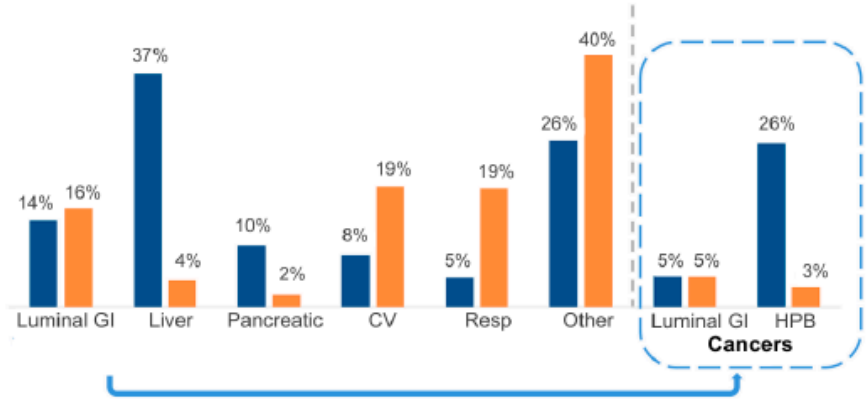
83% IBD then PSC
17% PSC then IBD




**INCIDENT COHORT MORTALITY**

- PSC-IBD: 30% mortality, median 65 y.o.
- IBD: 8.6% mortality, median 74 y.o.

**Predominant Underlying Cause of Death**




Leung et al. J Hep Reports 2024

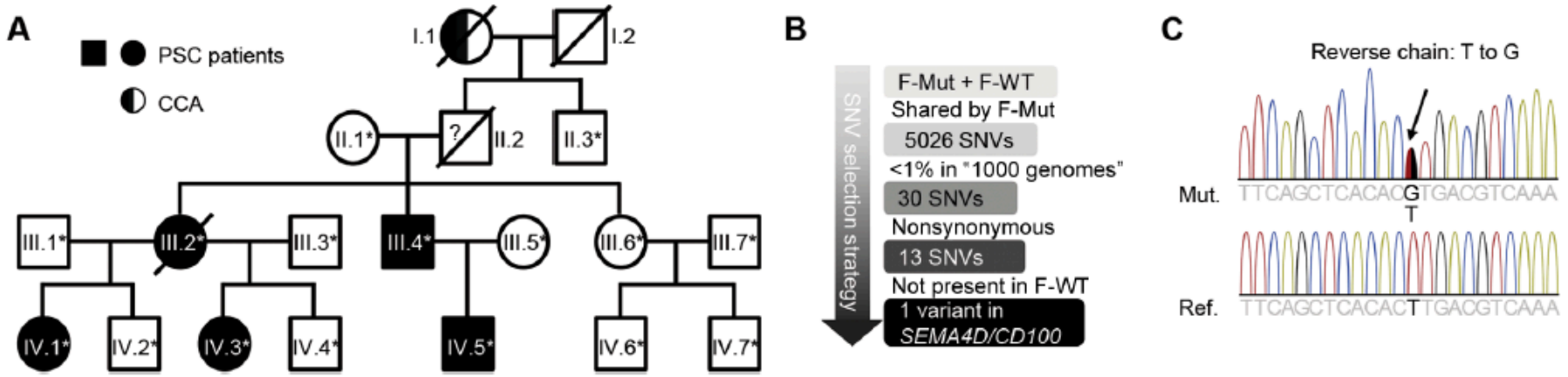
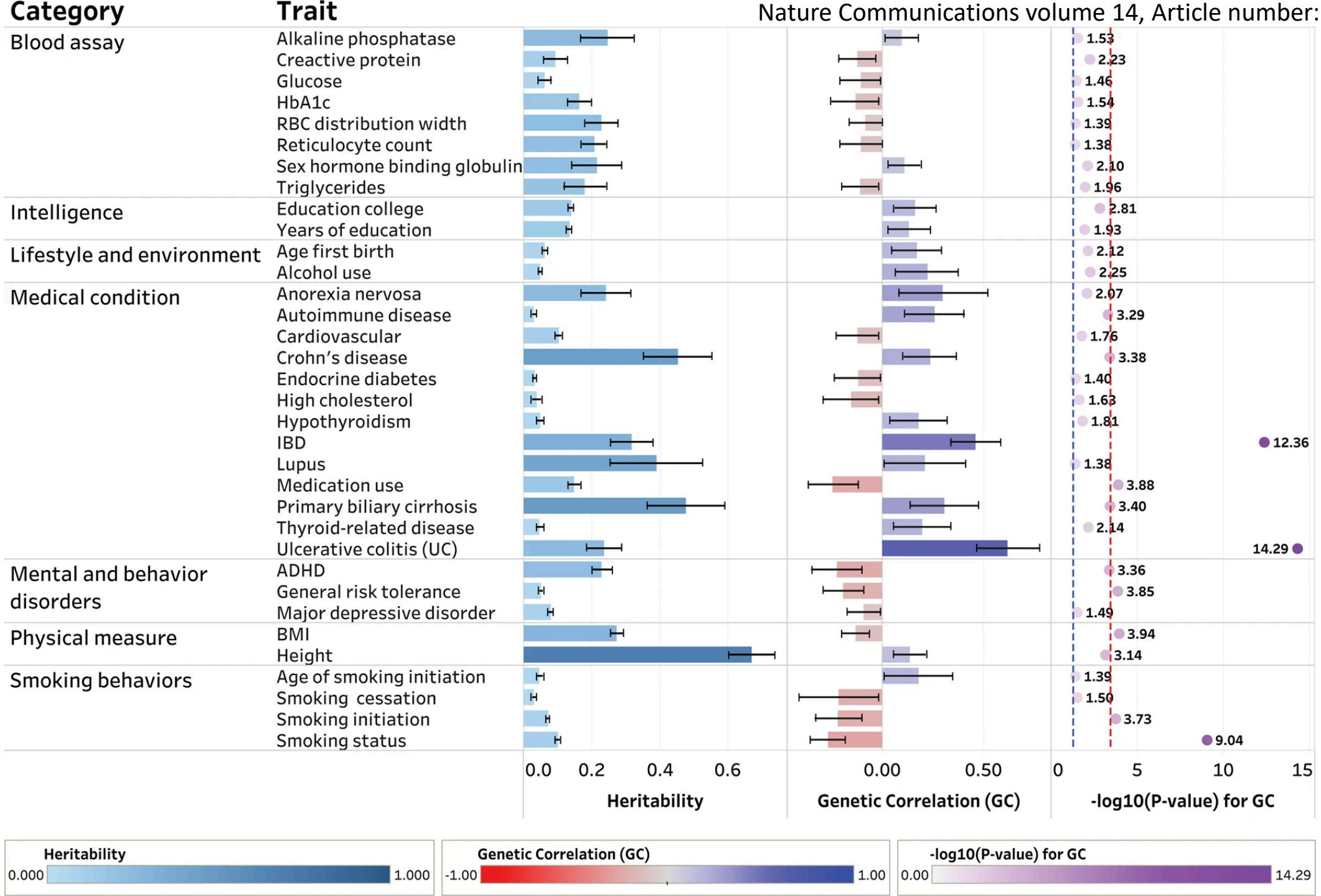



**Fig. 1. Identification of a missense mutation in *SEMA4D/CD100* in a family with PSC.** (**A**) Pedigree of a family with PSC. Squares, male participants; circles, female participants; black filled symbols, patients with PSC; half-filled symbol, patient with cholangiocarcinoma (CCA) but without a confirmed diagnosis of PSC; crossed-out symbols, deceased participants. Whole-exome sequencing was carried out on participants with an asterisk. (**B**) Single-nucleotide variant (SNV) selection strategy. (**C**) Confirmation of the $CD100^{K849T}$ mutation by Sanger sequencing. F-Mut, family members with PSC; F-WT, healthy family members.

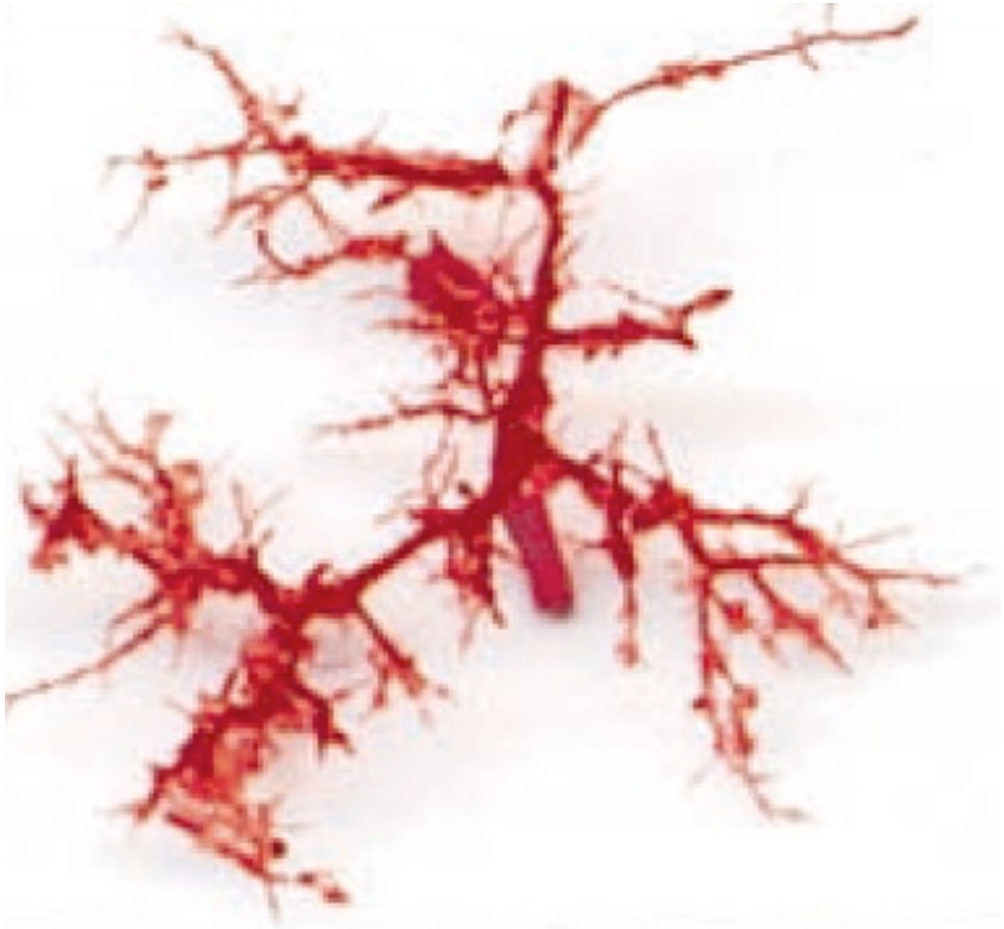
*"However, this mutation is not a common risk factor for PSC in general because our examination of 3178 patients did not identify any other carriers, and, to the best of our knowledge, it has not been reported in PSC elsewhere."*

Jiang et al., Sci. Transl. Med.13, eabb0036 (2021)

Nature Communications volume 14, Article number: 1069 (2023)



# Mdr2 deficient *mice develop* cholangiopathy



Gastroenterology 2002;123:1238–1251

Article

# Bile acid metabolites control $T_H17$ and $T_{reg}$ cell differentiation

https://doi.org/10.1038/s41586-019-1785-z

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Accepted: 17 September 2019

Published online: 27 November 2019

Saiyu Hang[1,12], Donggi Paik[1,12], Lina Yao[2], Eunha Kim[1], Trinath Jamma[3], Jingping Lu[4], Soyoung Ha[1], Brandon N. Nelson[5], Samantha P. Kelly[5], Lin Wu[6], Ye Zheng[7], Randy S. Longman[8], Fraydoon Rastinejad[4], A. Sloan Devlin[2], Michael R. Krout[5], Michael A. Fischbach[9]*, Dan R. Littman[6,10]* & Jun R. Huh[1,11]*

Bile acids are abundant in the mammalian gut, where they undergo bacteria-mediated transformation to generate a large pool of bioactive molecules. Although bile acids are known to affect host metabolism, cancer progression and innate immunity, it is unknown whether they affect adaptive immune cells such as T helper cells that express IL-17a ($T_H17$ cells) or regulatory T cells ($T_{reg}$ cells). Here we screen a library of bile acid metabolites and identify two distinct derivatives of lithocholic acid (LCA), 3-oxoLCA and isoalloLCA, as T cell regulators in mice. 3-OxoLCA inhibited the differentiation of $T_H17$ cells by directly binding to the key transcription factor retinoid-related orphan receptor-γt (RORγt) and isoalloLCA increased the differentiation of $T_{reg}$ cells through the production of mitochondrial reactive oxygen species (mitoROS), which led to increased expression of FOXP3. The isoalloLCA-mediated enhancement of $T_{reg}$ cell differentiation required an intronic *Foxp3* enhancer, the conserved noncoding sequence (CNS) 3; this represents a mode of action distinct from that of previously identified metabolites that increase $T_{reg}$ cell differentiation, which require CNS1. The administration of 3-oxoLCA and isoalloLCA to mice reduced $T_H17$ cell differentiation and increased $T_{reg}$ cell differentiation, respectively, in the intestinal lamina propria. Our data suggest mechanisms through which bile acid metabolites control host immune responses, by directly modulating the balance of $T_H17$ and $T_{reg}$ cells.

Article

# Human gut bacteria produce $T_H17$-modulating bile acid metabolites

https://doi.org/10.1038/s41586-022-04480-z

Received: 4 December 2020

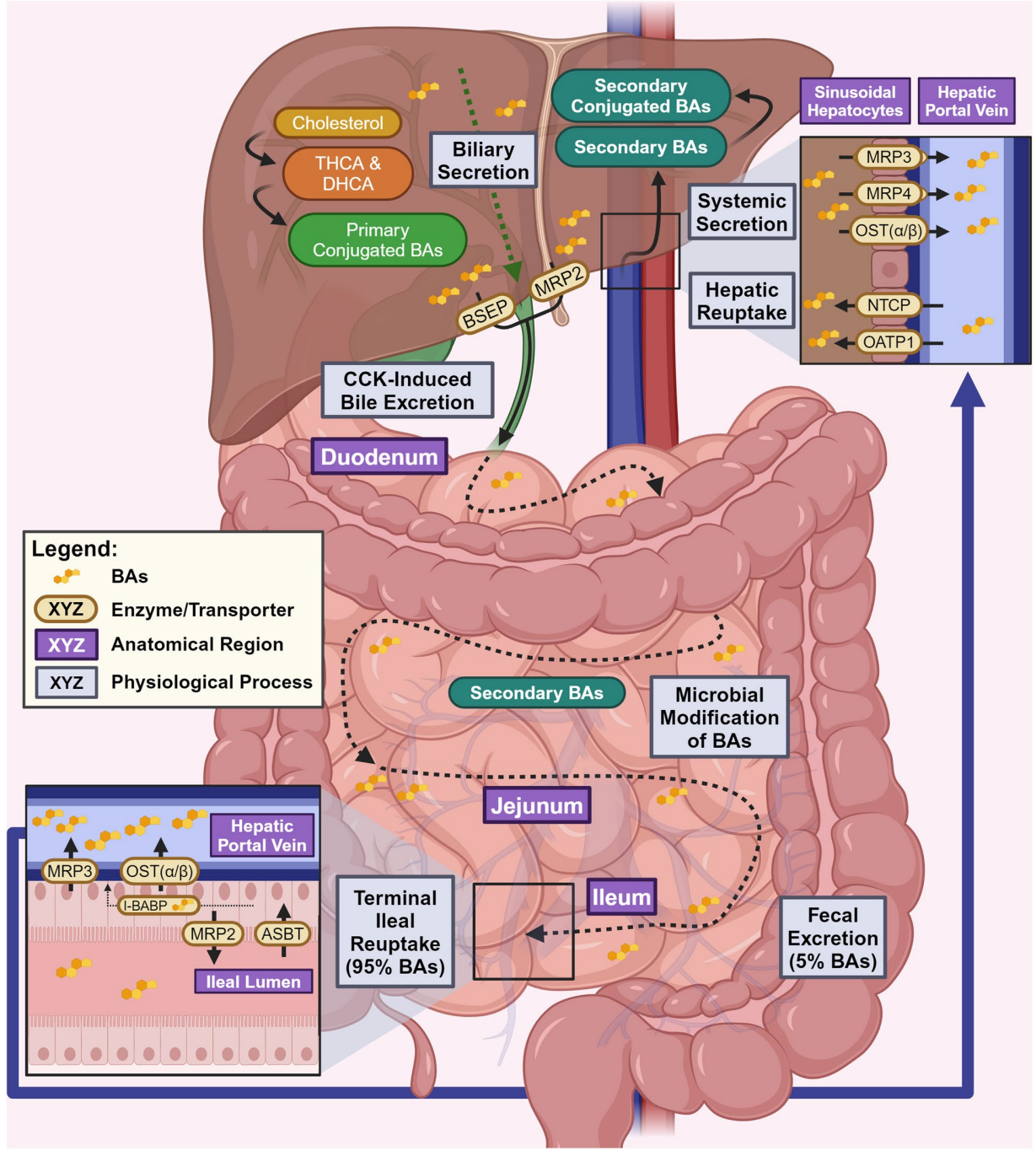
Accepted: 27 January 2022

Published online: 16 March 2022

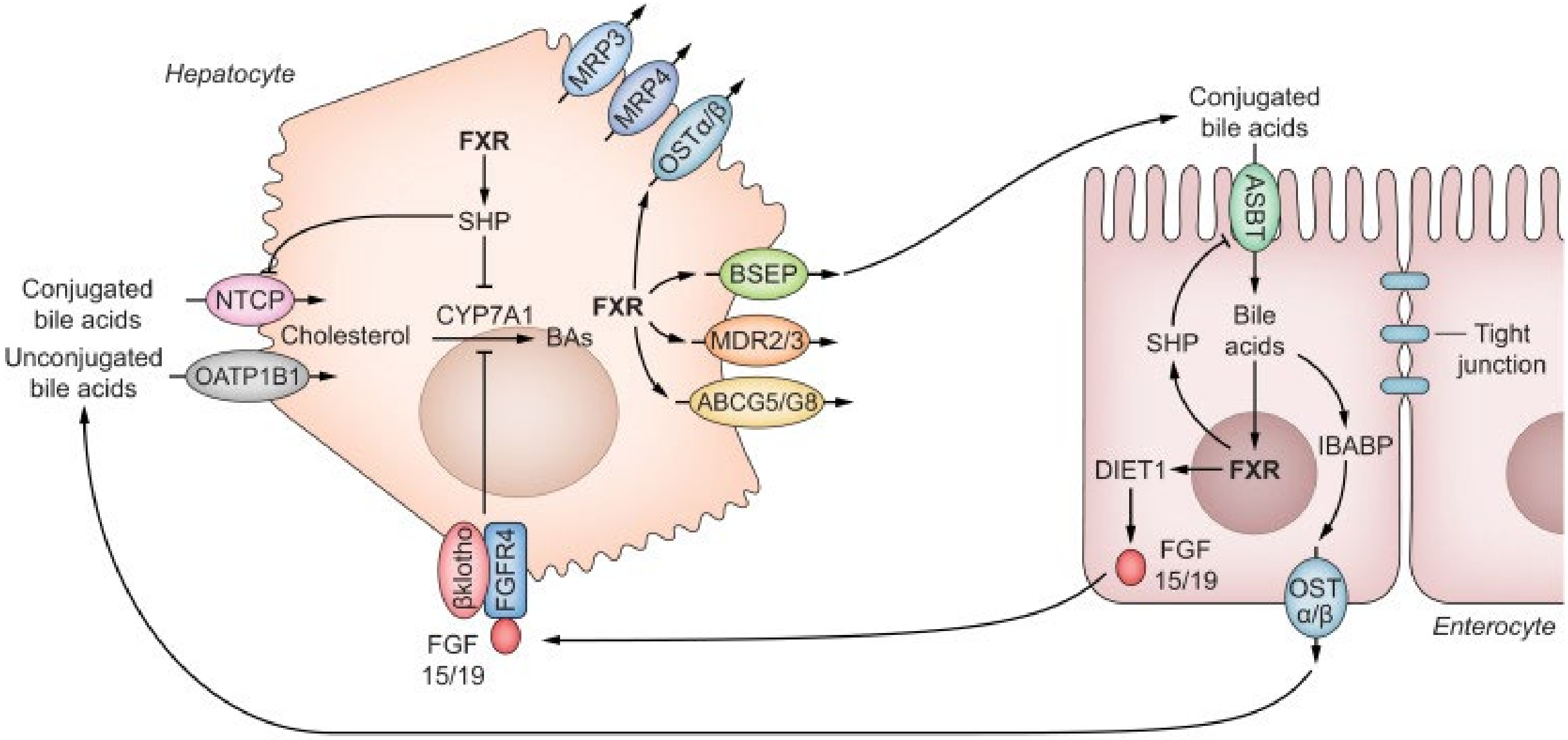
Check for updates

Donggi Paik[1,15], Lina Yao[2,15], Yanqong Zhang[3,4], Sena Bae[4,5], Gabriel D. D'Agostino[2], Minghao Zhang[6], Eunha Kim[1], Eric A. Franzosa[4,5], Julian Avila-Pacheco[7], Jordan E. Bisanz[7], Christopher K. Rakowski[8], Hera Vlamakis[3,9], Ramnik J. Xavier[3,9,10,11], Peter J. Turnbaugh[7,12], Randy S. Longman[13], Michael R. Krout[8], Clary B. Clish[3], Fraydoon Rastinejad[6], Curtis Huttenhower[3,4,5], Jun R. Huh[1,14,16] & A. Sloan Devlin[2,16]

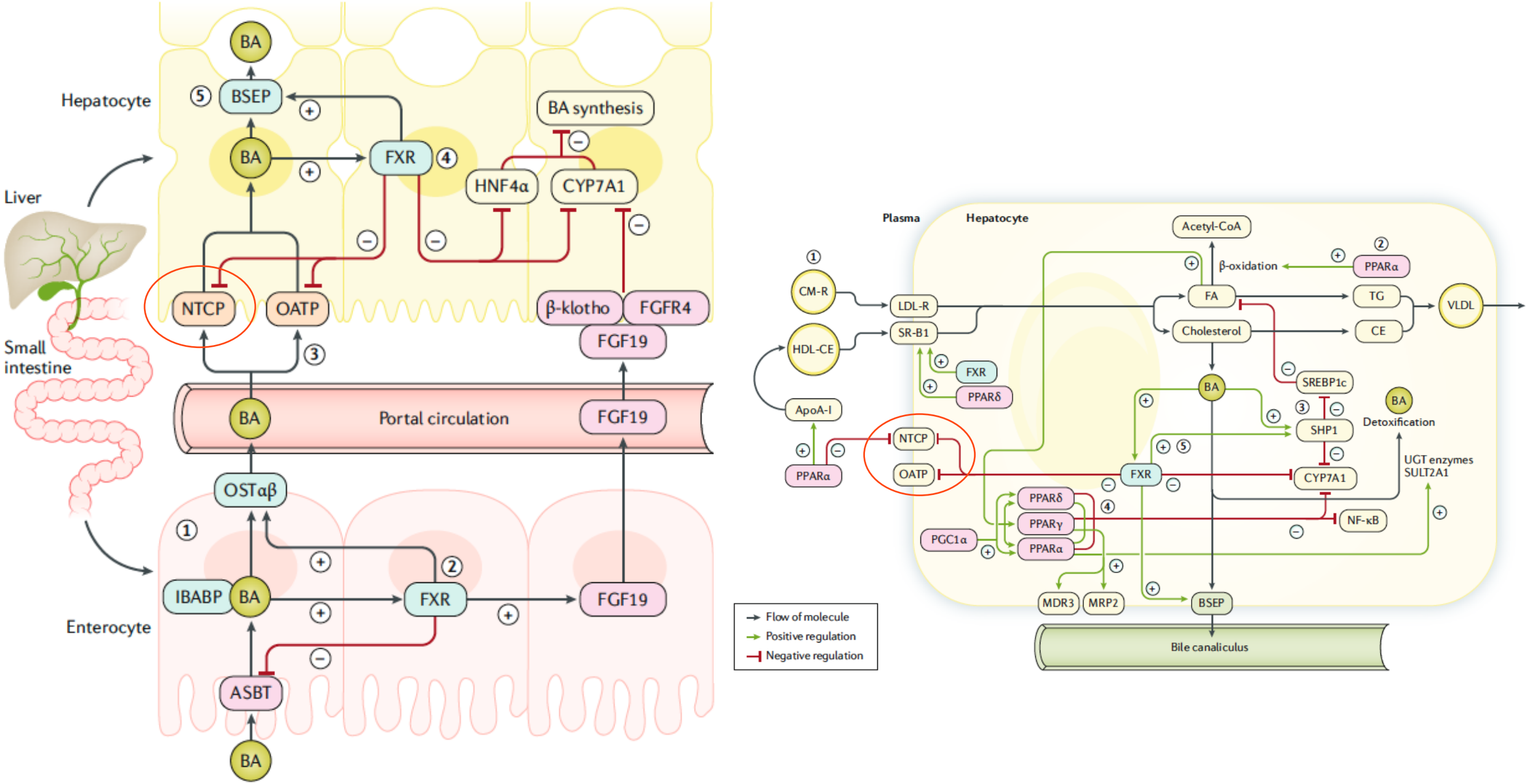
The microbiota modulates gut immune homeostasis. Bacteria influence the development and function of host immune cells, including T helper cells expressing interleukin-17A ($T_H17$ cells). We previously reported that the bile acid metabolite 3-oxolithocholic acid (3-oxoLCA) inhibits $T_H17$ cell differentiation[1]. Although it was suggested that gut-residing bacteria produce 3-oxoLCA, the identity of such bacteria was unknown, and it was unclear whether 3-oxoLCA and other immunomodulatory bile acids are associated with inflammatory pathologies in humans. Here we identify human gut bacteria and corresponding enzymes that convert the secondary bile acid lithocholic acid into 3-oxoLCA as well as the abundant gut metabolite isolithocholic acid (isoLCA). Similar to 3-oxoLCA, isoLCA suppressed $T_H17$ cell differentiation by inhibiting retinoic acid receptor-related orphan nuclear receptor-γt, a key $T_H17$-cell-promoting transcription factor. The levels of both 3-oxoLCA and isoLCA and the 3α-hydroxysteroid dehydrogenase genes that are required for their biosynthesis were significantly reduced in patients with inflammatory bowel disease. Moreover, the levels of these bile acids were inversely correlated with the expression of $T_H17$-cell-associated genes. Overall, our data suggest that bacterially produced bile acids inhibit $T_H17$ cell function, an activity that may be relevant to the pathophysiology of inflammatory disorders such as inflammatory bowel disease.



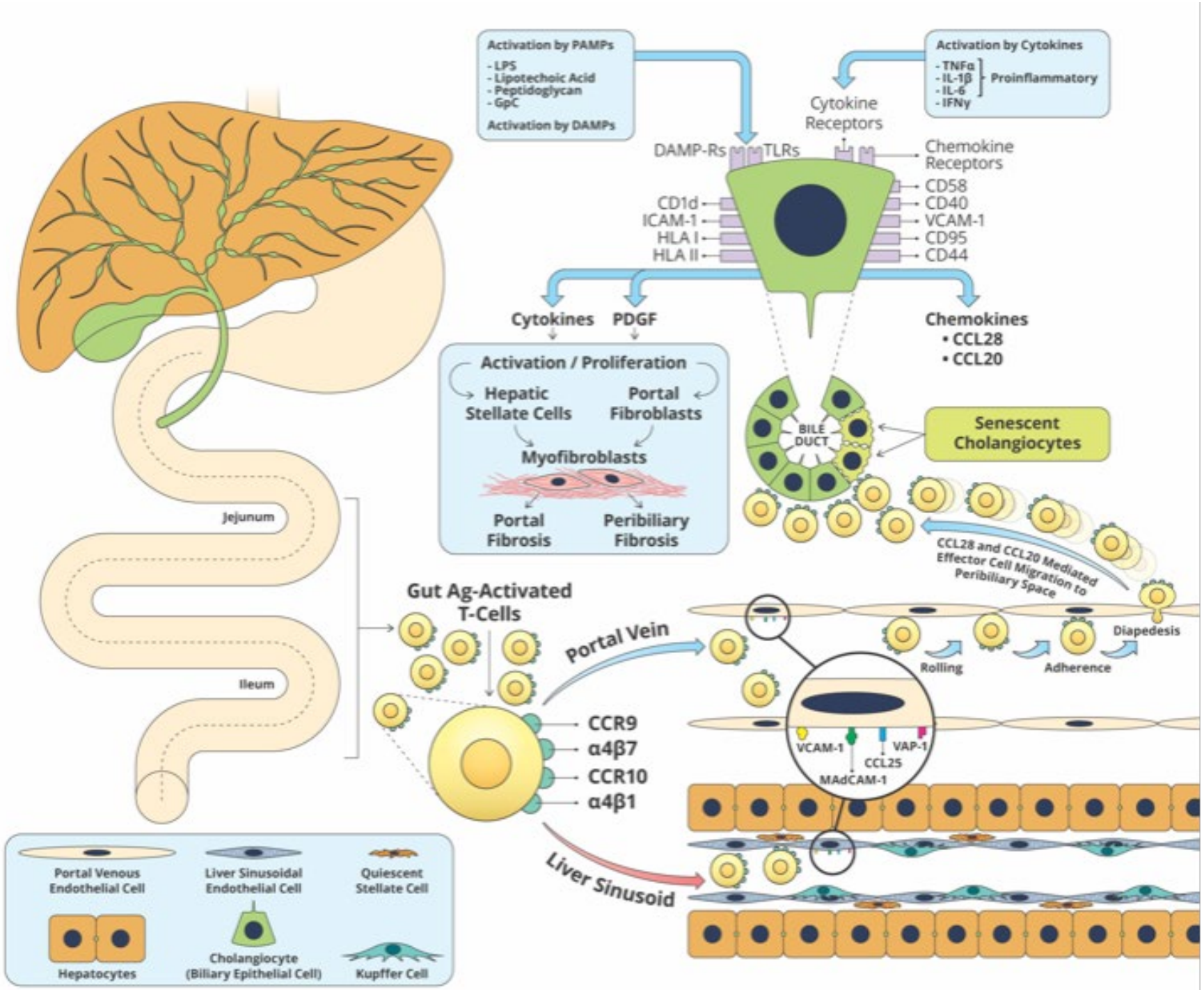

**Signal Transduction and Targeted Therapy (*Sig Transduct Target Ther*)** ISSN 2059-3635 (online)




Fuchs et al J Hep 2024




**Gulamhusein and Hirschfield Nat Rev Gastro Hep 2020**




Trivedi, Hirschfield, Adams, Vierling
Gastroenterology 2024

## C COVERT

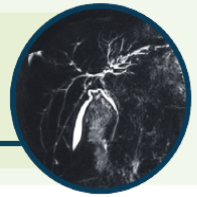
PSC affects both sexes and occurs at all ages; however, the majority of patients are male and the median age at onset is 30–40 years. Up to 80% of cases are associated with IBD. Approximately 50% of patients with PSC are asymptomatic at diagnosis.

### PSC SYMPTOMS

When symptomatic, PSC is insidious. Patients most often complain of abdominal pain, pruritus, and fatigue.

### PSC DIAGNOSIS

Diagnosis is usually based on: ❶ Serum ALP elevation, ❷ multi-focal biliary strictures with intervening dilatations on cholangiography (usually MRCP), ❸ exclusion of secondary sclerosing cholangitis, and ❹ liver biopsy when small-duct PSC or PSC-AIH is suspected.



Typical MRCP in PSC

### HOLISTIC APPROACH

PSC care must integrate disease monitoring, treatment and research with psychosocial support that addresses the fear, uncertainty, and social isolation many patients experience. PSC support societies are an excellent resource.


## C CHOLANGITIS

Genetic and environmental factors interact to establish the pathogenesis of PSC, which involves the gut microbiota, impaired bile acid composition and cholestatis, and autoimmunity.

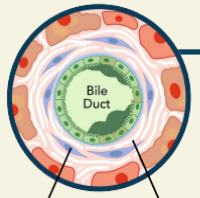
**GENETICS**
> 20 HLA and non-HLA loci have been linked to PSC, establishing it as an autoimmune disease.

**MICROBIOTA**
Altered gut and biliary microbiota may drive the immune response in PSC.

**IMMUNE RESPONSE**
The predominant cells identified in the vicinity of bile ducts are T cells, macrophages and neutrophils.

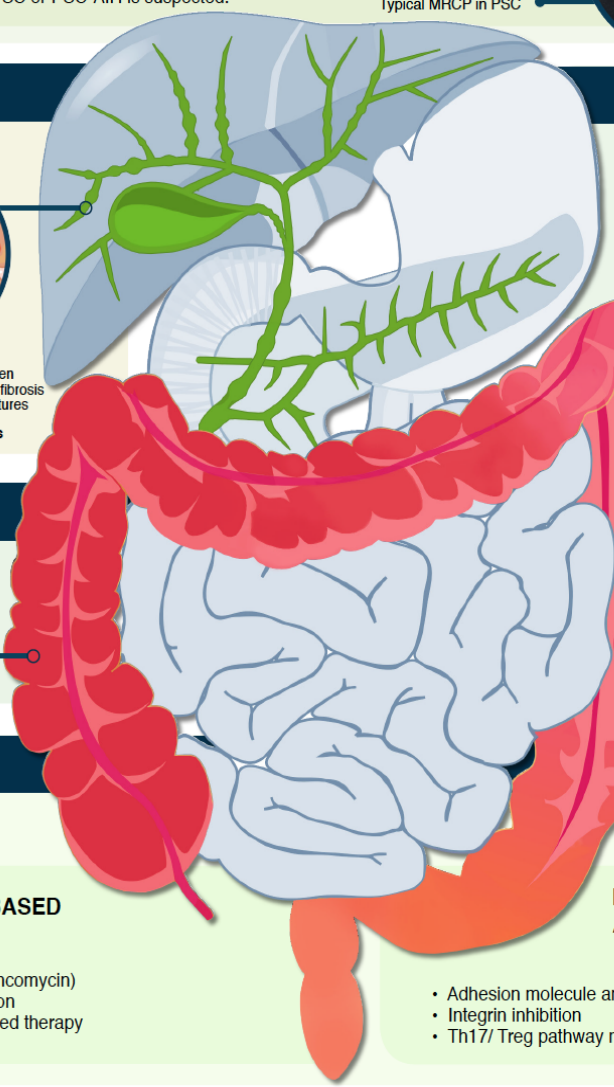
**ENVIRONMENT**
Multiple environmental exposures have been associated with PSC.

**BILE ACIDS**
Bile acid homeostasis is impaired and biliary epithelium is activated.

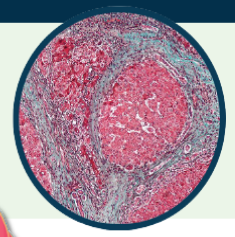



Activated fibroblasts and stellate cells (not shown) → Collagen deposition, fibrosis and strictures

"Onion-skinning" fibrosis



## CIRRHOSIS C



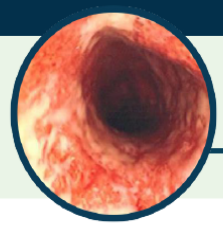
The end-point of PSC is cirrhosis.

The extent of inflammation and fibrosis observed does not necessarily correlate with the risk of biliary dysplasia or malignancy.

## C COLITIS

**PSC-IBD is phenotypically distinct from IBD without PSC.**

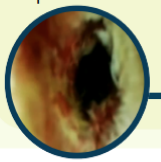
- Majority of IBD in PSC patients is UC and presents earlier than in those without PSC
- Frequently presents with pancolitis, predominantly right-sided, with "back-wash ileitis" and rectal sparing



## CANCER C

### CANCER RISK

PSC patients are at increased risk of **colorectal** and **hepatopancreatobiliary cancers**, including cholangiocarcinoma, hepatocellular carcinoma, pancreatic cancer, and gallbladder cancer.



Cholangiocarcinoma

### PSC SURVEILLANCE

- Colonoscopy with screening biopsies at diagnosis and every 1-2 years
- Annual US
- Annual MRI/MRCP

Non-cancer screening:

- If cirrhosis: US and AFP every 6 months, screening for complications per guidelines
- Screening for osteoporosis and malnutrition

## C CURE

Liver transplantation is indicated per regional guidelines, including for decompensated cirrhosis, intractable pruritus, recurrent bacterial cholangitis, and HCC.

- 5-year survival post-transplantation exceeds 80%
- PSC recurrs at a rate of approximately 20% post-transplantion

### BILE ACID-BASED THERAPY

- UDCA and experimental analogues
- *Nor*UDCA
- FXR and FGF19 analogues
- PPAR agonists
- ASBT inhibitors

### MICROBIOTA-BASED THERAPY

- Antibiotics (e.g. vancomycin)
- Fecal transplantation
- Bacteriophage-based therapy

### IMMUNE-MODULATING & ANTI-FIBROTIC THERAPY

- Adhesion molecule and chemokine inhibition
- Integrin inhibition
- Th17/ Treg pathway modification

### BIOMARKERS

Biomarkers are important for prognostication & evaluating treatment effect.

- ?ALP
- ?ELF
- ?PRO-C3
- Liver biopsy
- Quantitative MRI
- Elastography

**Deeb et al. J Hep 2020**

# THE AUTOIMMUNE & RARE LIVER DISEASE PROGRAMME

PBC, PSC, AIH, Hepato-biliary IgG4-RD & Genetic Cholestasis

**Care** **Teaching** **Research**

# THANK YOU

**Gideon Hirschfield**

# AX-0810 RNA editing therapy targeting NTCP for cholestatic diseases

Cholestatic diseases have high unmet medical need. Patients accumulate bile acids in liver leading to fibrosis and ultimately liver failure.

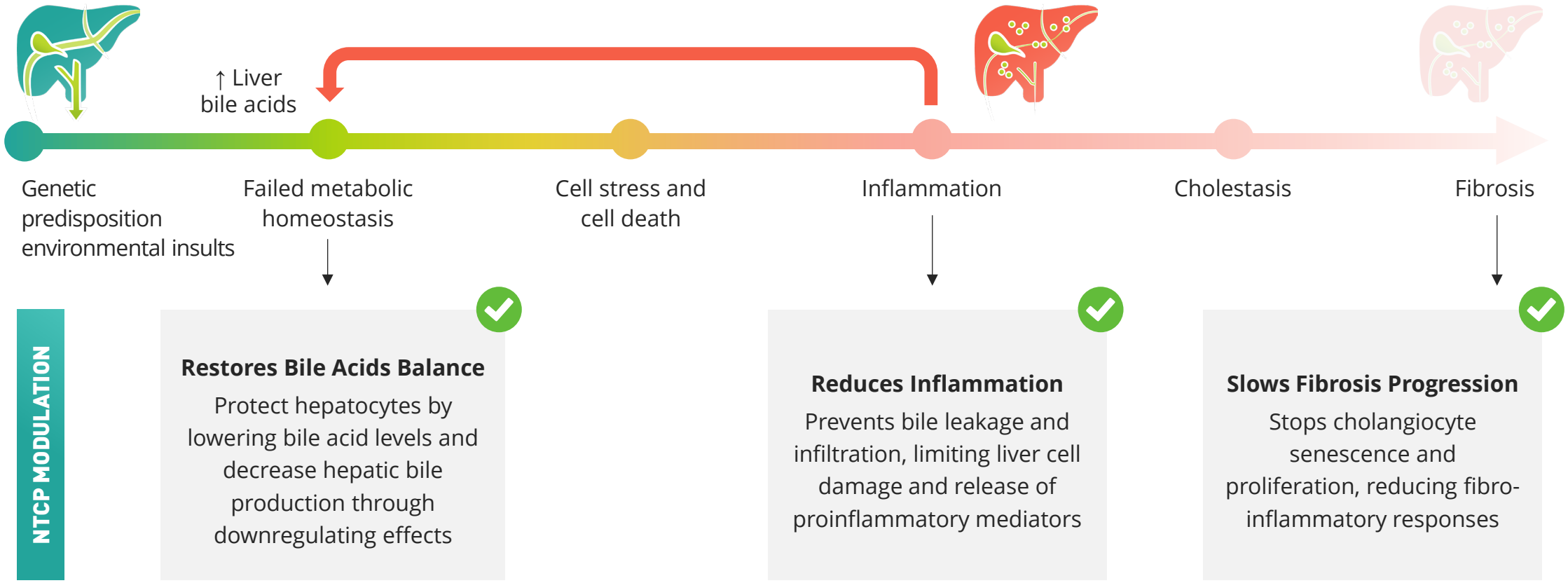
Initial indications are **Primary Sclerosing Cholangitis** affecting adults and Congenital **Biliary Atresia** affecting pediatrics early in life. Both conditions have no approved therapies and may require liver transplantation.[1,2]

- **Biliary Atresia** is projected to affect ~20,000 pediatric individuals in US and EU.
- **Primary Sclerosing Cholangitis** is projected to affect more than 80,000 individuals in US and EU.

AX-0810 is a unique therapeutic approach leading to a potentially disease modifying therapy by targeting the NTCP channel which is responsible for majority of bile acid re-uptake in liver cells.


[1]Trivedi PJ, et al. Clin Gastroenterol Hepatol. 2022 Aug;20(8):1687-1700.e4; [2]Schreiber RA, et al. J Clin Med. 2022 Feb 14;11(4):999

# NTCP modulation leads to positive effect on different mechanism involved in cholestasis




Zeng J, Fan J, Zhou H. Cell Biosci. 2023 Apr 29;13(1):77; Trauner M, Fuchs CD. Gut 2022;71:194–209; Halilbasic E, Claudel T, Trauner M. J Hepatol. 2013 Jan;58(1):155-68.

# NTCP variants reduced bile acids uptake into liver in health population research

Healthy population discovered with NTCP variants that reduces bile acids uptake into liver[1-4]

Ethnicity-dependent Polymorphism in Na+-taurocholate Cotransporting Polypeptide (*SLC10A1*) Reveals a Domain Critical for Bile Acid Substrate Recognition*

Richard H. Ho‡§¶, Brenda F. Leake‡, Richard L. Roberts‖, Wooin Lee‡, and Richard B. Kim‡**

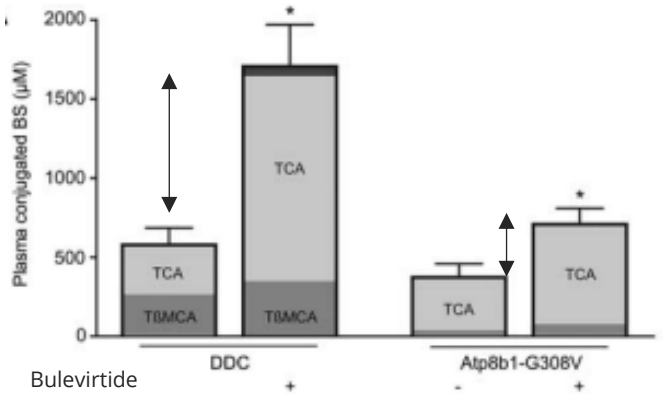
d D Viruses and Bile Salts
on Molecular Determinants on Sodium
Polypeptide

[1]Salhab A, et al. Gut. 2022 Jul;71(7):1373-1385; [2]Ho RH, et al. J Biol Chem. 2004 Feb 20;279(8):7213-22; [3]Vaz FM, et al. Hepatology. 2015 Jan;61(1):260-7; [4]Schneider AL, et al. Clin Res Hepatol Gastroenterol. 2022 Mar;46(3):101824; [5]Slijepcevic D, et al. Hepatology. 2018 Sep;68(3):1057-1069; [6]Cai SY, et al. JCI Insight. 2017 Mar 9;2(5):e90780.

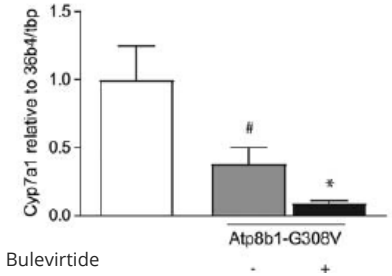
# NTCP modulation has hepato-protective effects *in vivo* in disease models

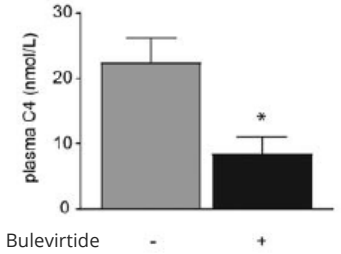
**3-fold** increase in conjugated BA

**2-fold** increase in conjugated BA

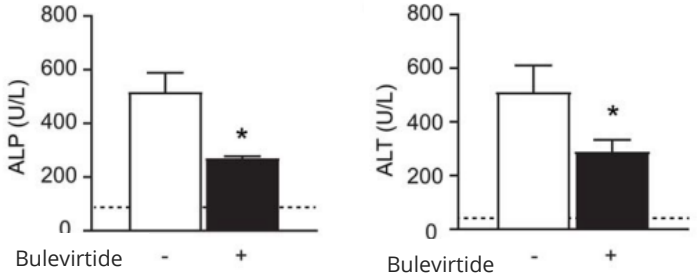


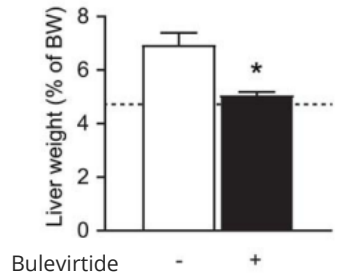

NTCP inhibition **increases plasma bile acids concentrations**
(2- to 3-fold in mouse models)




**Reduced bile acid production** during cholestasis (expected to decrease intrahepatic bile acids load)



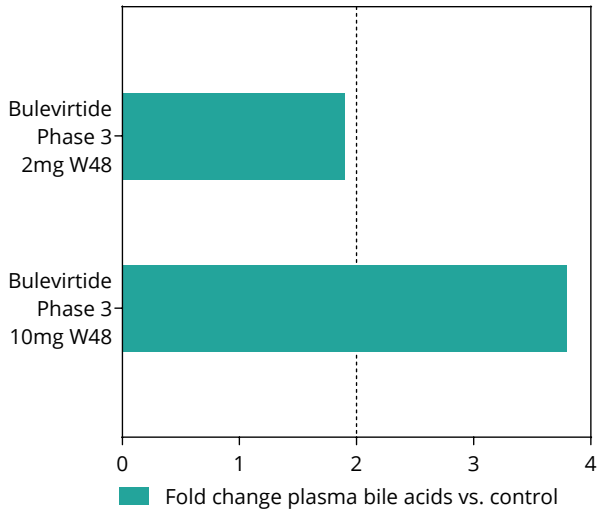




Reduced cholestatic liver injury via **improvement in liver enzymes**

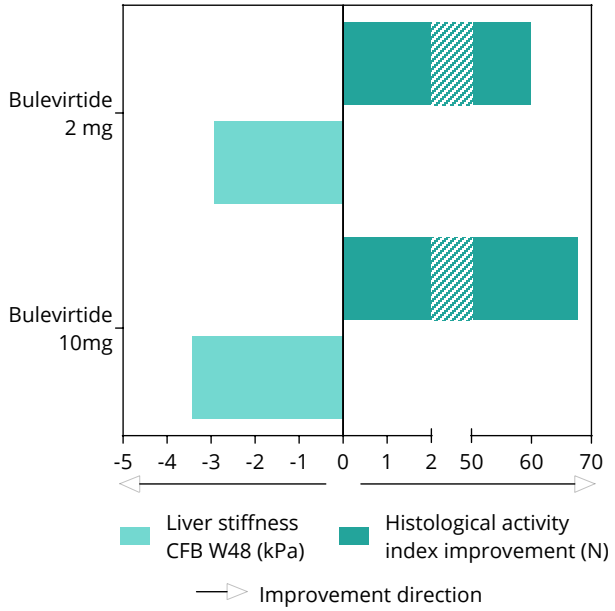
Bulevirtide (Hepcludex) is a daily SC injected NTCP inhibitor approved for Hepatitis D. Slijepcevic D, et al. Hepatology. 2018 Sep;68(3):1057-1069.

# NTCP modulation leads to clinically meaningful impact in patients

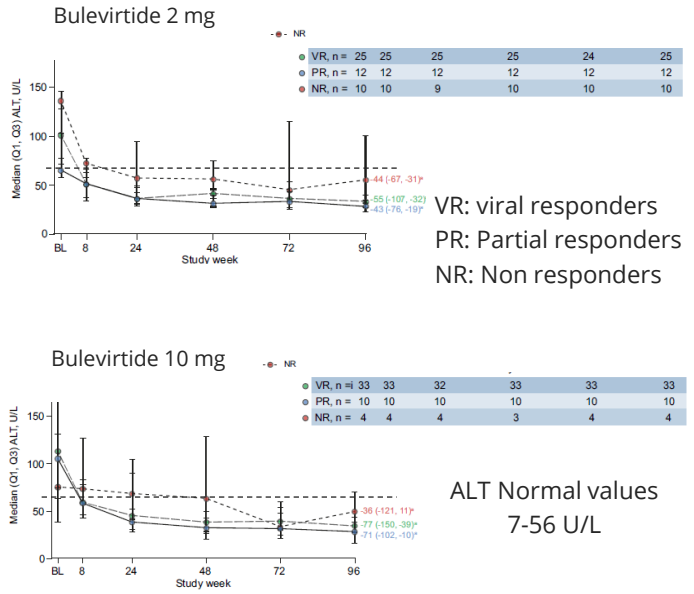
**Reducing liver bile acids toxic overload via NTCP modulation is a key driver for hepatoprotective effects**




NTCP inhibition **increases plasma bile acids concentrations in humans (2- to 4-fold)**



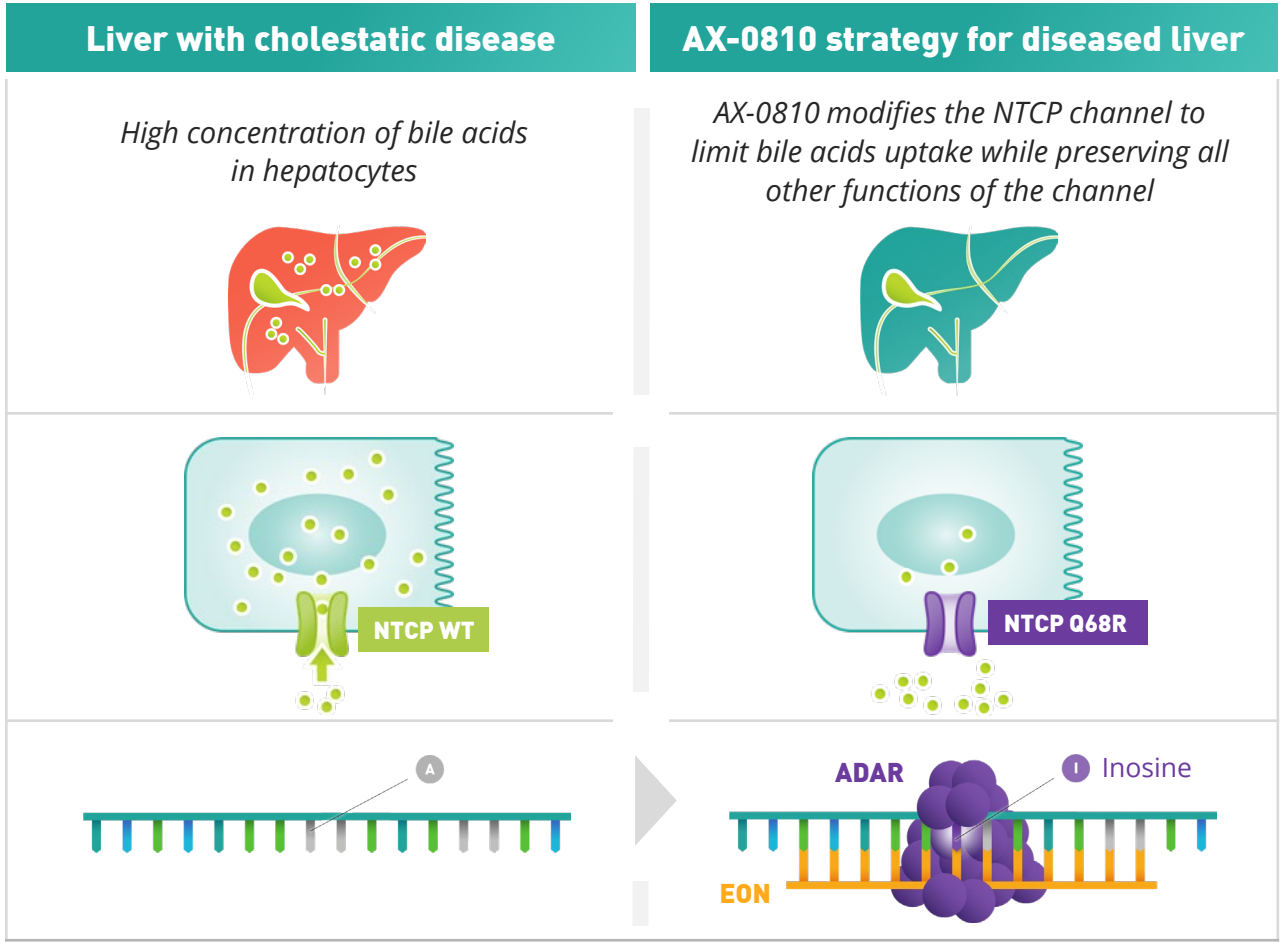

Treatment with NTCP inhibitor led to **improvement in liver fibrosis (stiffness and histology)**




**Liver enzyme improvement** occur in patients, **even without virologic response***

*NTCP channel is a known transporter for bile acids and hepatitis virus from bloodstream to the liver. Bulevirtide (Hepcludex) is a daily SC injected NTCP inhibitor approved for Hepatitis D. Wedemeyer H, et al. N Engl J Med. 2023 Jul 6;389(1):22-32; Wedemeyer H, J Hepatol. 2024 Oct;81(4):621-629.; Dietz-Fricke C, JHEP Rep. 2023 Mar 15;5(4):100686.

# Human genetics validates NTCP modulation as strategy for cholestatic disease

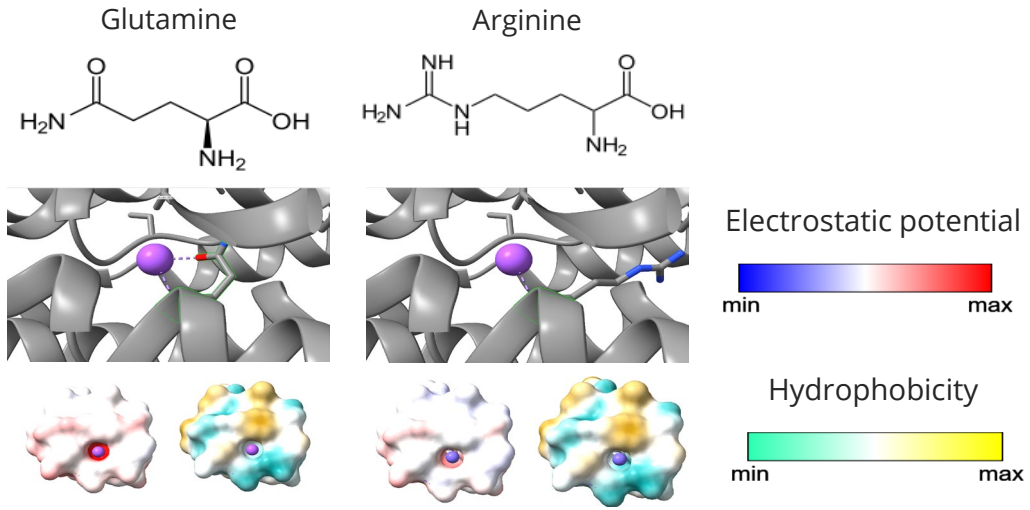



- The AX-0810 program introduces a variant in individuals with cholestatic disease to lower bile acids concentration in hepatocytes by a single A-to-I change
- The AX-0810 program is designed to be a disease modifying treatment
  - To alleviate symptoms in PSC and BA
  - To limit inflammation and fibrosis linked to bile acid toxicity
  - To prevent or delay the development of cirrhosis, organ failure and need for transplant

BA, Biliary atresia; PSC, Primary Sclerosing Cholangitis

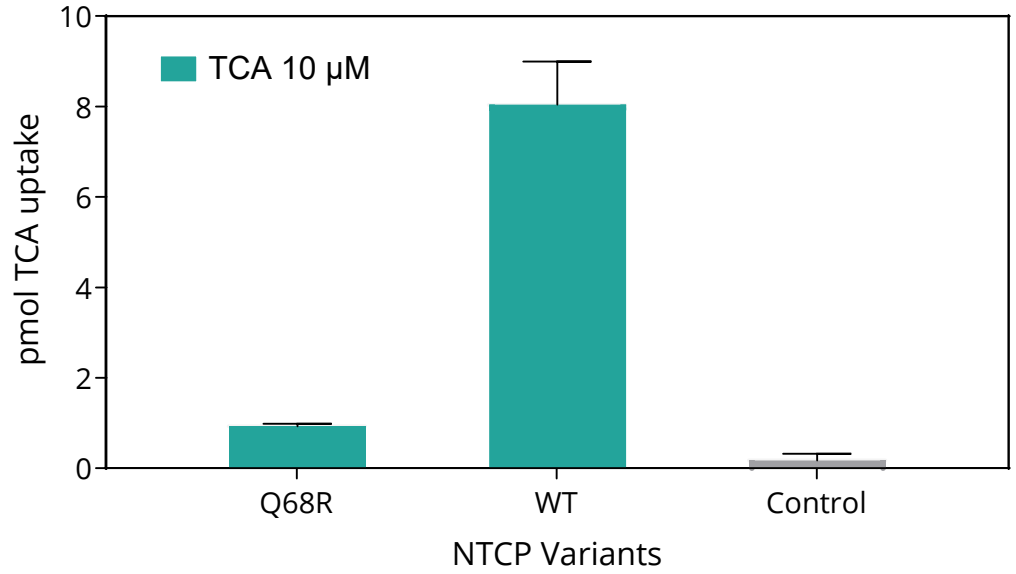
# Q68R NTCP variant leads to modulation of bile acids re-uptake

**3D Model of Q68R variant impact on $Na^+$ binding pocket of NTCP**




**BAs uptake (TCA) *in vitro**

*n=3, mean±SEM*



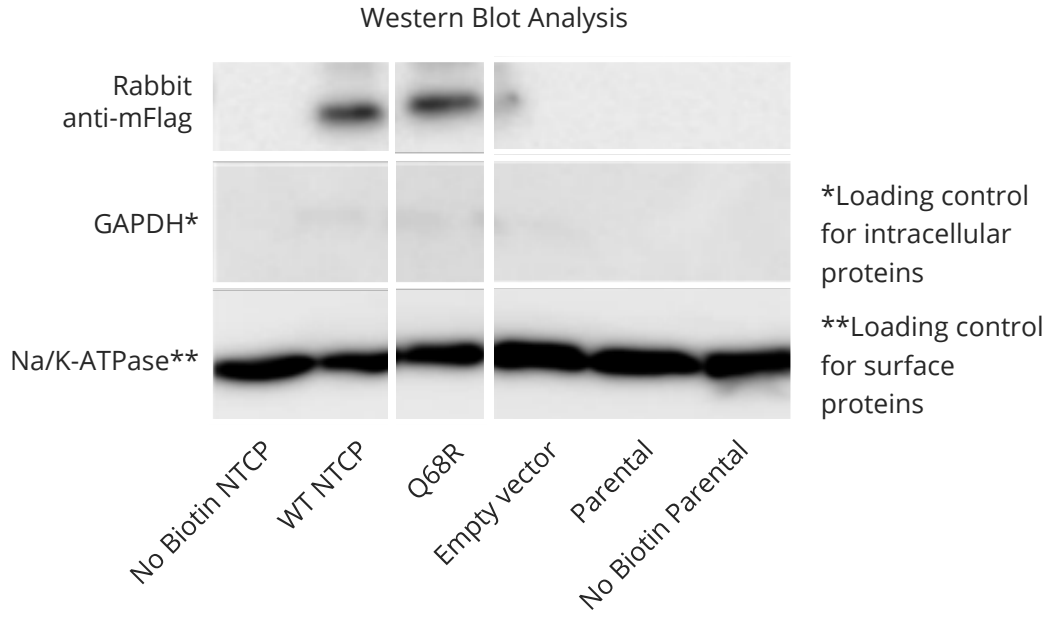

- The Q68R variant disrupts some hydrogen bonds and contacts in the $Na^+$ binding pocket.
- Clashes are inevitable since the Arg side chain is buried and likely to be found in one or another unfavorable rotamer state.

- Further assessment of Q68R variant in a bile acids uptake assay showed a near complete inhibition of BAs (specifically Taurocholic Acid or TCA) uptake *in vitro*, confirming findings from the 3D modeling

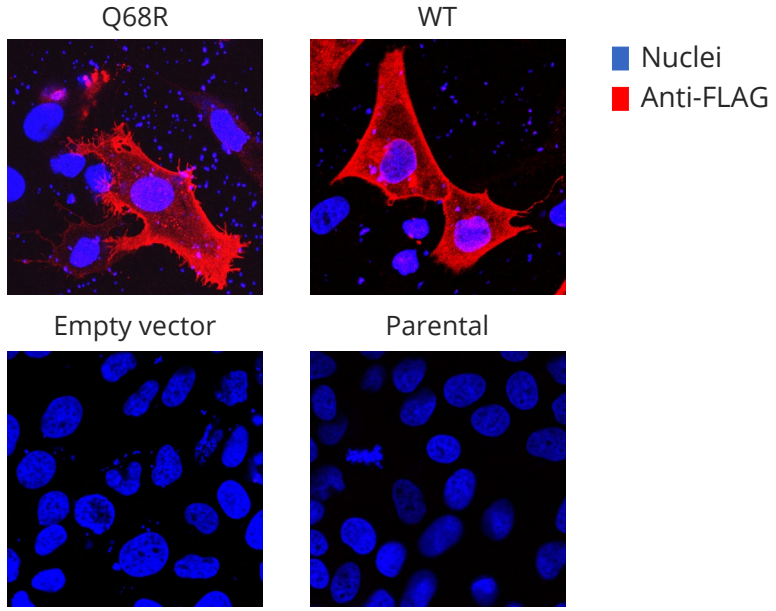
NTCP: Na-taurocholate cotransporting polypeptide, *Transiently transfected U2OS cells. Control is WT without TCA.

# Q68R NTCP variant solely affects bile acids re-uptake function

## NTCP protein expression was detected on western blot using the anti-FLAG antibody for all constructs




## NTCP protein localization in vitro*




- No significant differences in NTCP RNA and protein levels were detected. The plasma membrane location of the Q68R variant was also unaffected.
- The Q68R variant solely affects NTCP bile acids reuptake function making it an approach of interest for Axiomer EON therapeutic application.

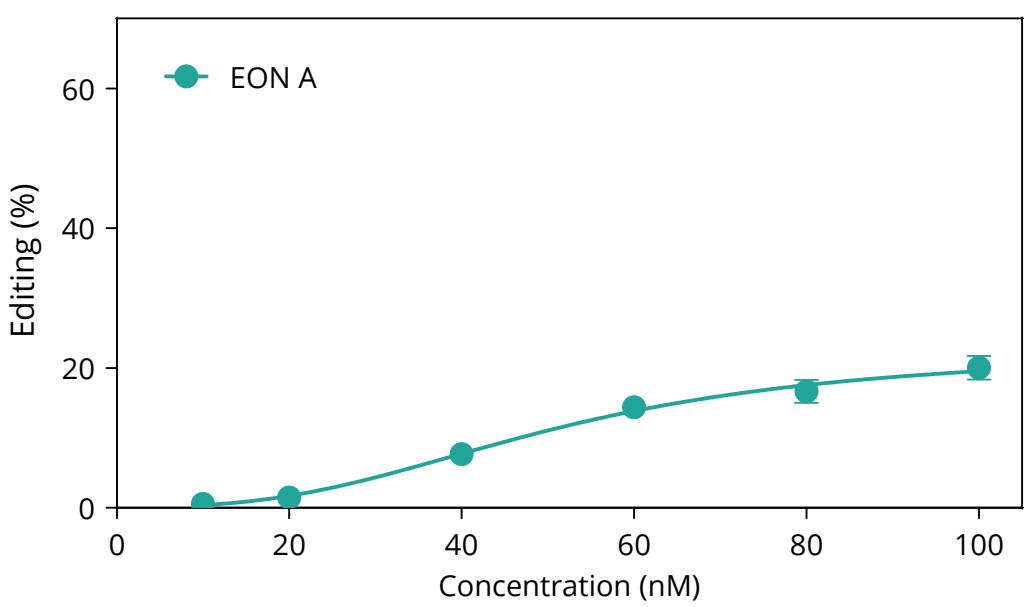
EON: editing oligonucleotide, NTCP: Na-taurocholate cotransporting polypeptide, *transiently transfected U2OS cells. S*LC10A1* is the gene that encodes for NTCP protein

# EON mediated RNA editing leads to NTCP Q68R variant in WT hepatocytes

*Editing of NTCP RNA modulates bile acids reuptake in a dose dependent fashion*

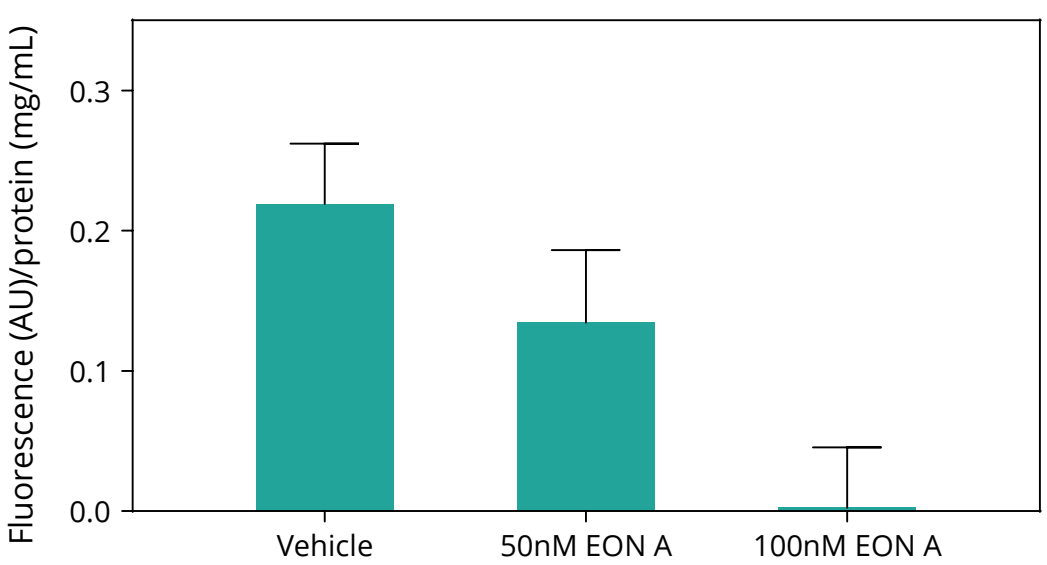
**Early generation of EONs targeting NTCP RNA in PHH**

*Transfection, n=3, 72 hours, mean±SEM, dPCR*




**NTCP-mediated BAs uptake in HepaRG cells with Axiomer EON treatment**

*n=3, 50-100nM, 72 hours, mean±SEM*



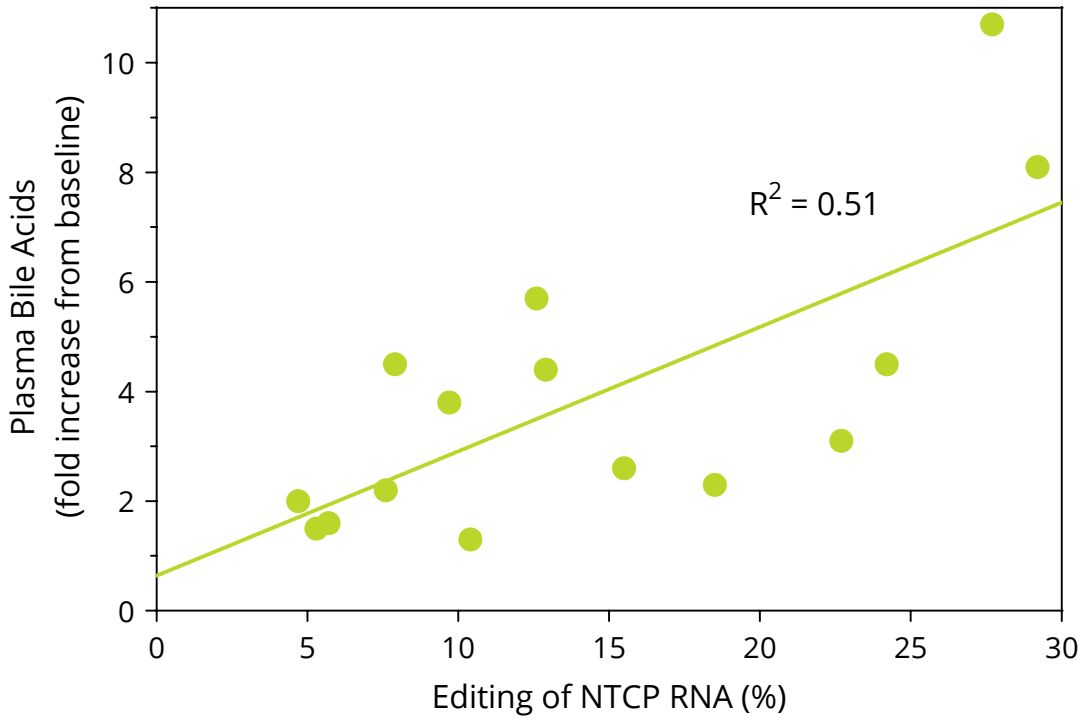

Early generation of EONs induces a dose-response inhibition of bile acids *in vitro* confirming its modulation by NTCP

NTCP: Na-taurocholate cotransporting polypeptide, BAs mentioned in this experiment are specifically Tauro-nor-THCA-24-DBD. *SLC10A1* is the gene that encodes for NTCP protein

# EON mediated NTCP editing in NHP has linear correlation with bile acids plasma levels

**Correlation between change in plasma BAs and editing of NTCP RNA in NHPs *in vivo***

*n=6, Early generation EONs, IV, LNP formulation, 72 hours, dPCR*




- NTCP target engagement with Axiomer EONs leads to the desired changes in biomarkers
- Correlation between plasma bile acids and early-generation EONs editing level in NHPs *in vivo* (linear regression $R^2$ = 0.51)

NTCP: Na-taurocholate cotransporting polypeptide, BAs mentioned in this experiment are specifically Tauro-nor-THCA-24-DBD. *SLC10A1* is the gene that encodes for NTCP protein

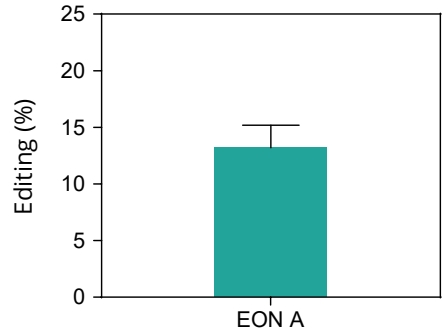
# EON mediated editing demonstrates consistent editing of NTCP and impact on biomarker *in vivo*

**MICE *in vivo***

**EDITING EFFICIENCY**

**NTCP RNA Editing in Humanized Mice**

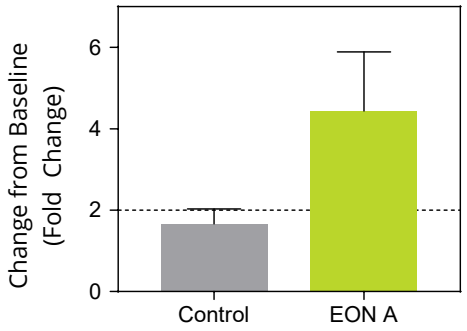
*(N=4, 20mg/kg, 6 doses, GalNAc conjugation, SC, D25, ddPCR)*




**PLASMA TOTAL BILE ACIDS**

**Plasma TBA in Humanized Mice**

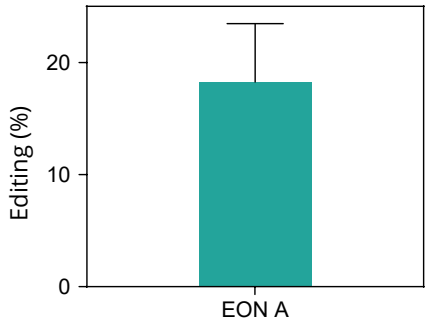
*(N=4, 20mg/kg, 6 doses, GalNac conjugation, SC, D25)*




**NHP *in vivo***

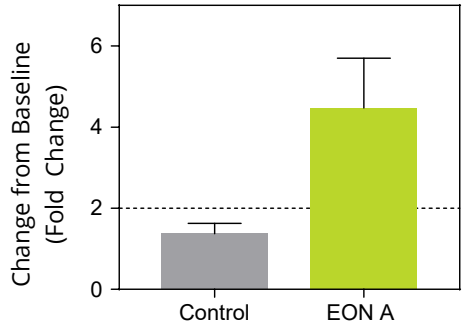
**NTCP RNA Editing in NHP**

*(N=1, 1-4mg/kg, 4 doses, LNP formulation, IV, up to D46, ddPCR)*




**Plasma TBA in NHP**

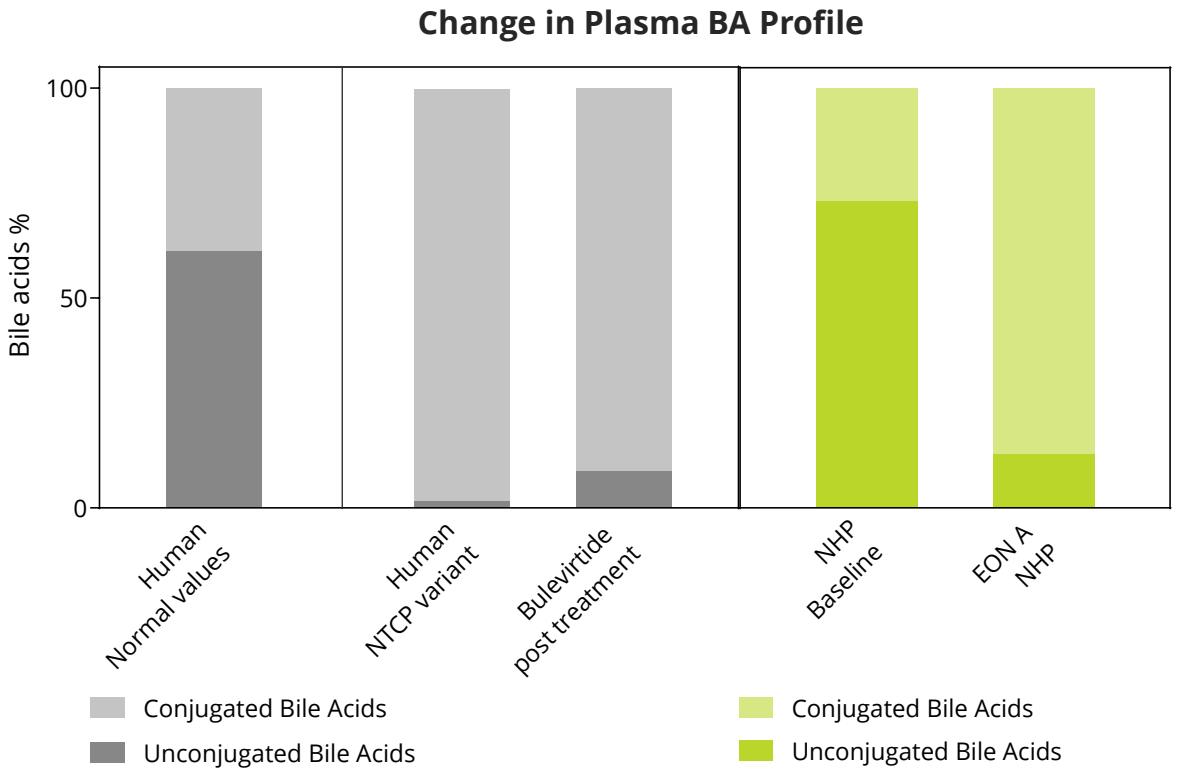
*(N=1, 1-4mg/kg, 4 doses, LNP formulation IV, up to D39)*




- EON A results in consistent editing data in humanized mouse model and NHP *in vivo* with approx. 15% editing reaching expected NTCP modulation
- Reaching >2-fold changes in biomarkers - expected impact on plasma bile acids levels following NTCP EON treatment

# NTCP editing demonstrates favorable composition of bile acids profile in NHP

*Increase in conjugated bile acids confirms NTCP engagement in vivo*



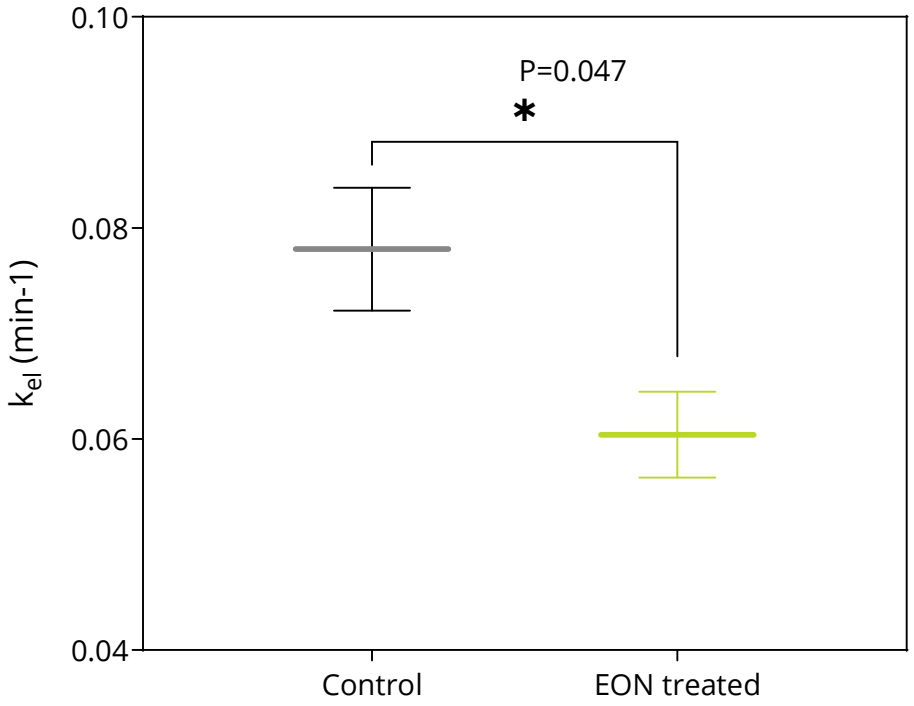

- Conjugated bile acids are transported by NTCP back to the liver
- The observed change in plasma BA profile confirms NTCP specific modulation
- In view of the preclinical data, high confidence on NTCP EON treatment to positively impact BA toxic load in the liver

Conditions in humanized mice: N=4, 20mg/kg, 6 doses, GalNAc conjugation, SC, D25, ddPCR; Conditions in the NHP experiment N=1, 1-4mg/kg, 4 doses, LNP formulation, IV, up to D42, ddPCR. Mao F, et al. J Biol Chem. 2019 Aug 2;294(31):11853-11862; Haag M, et al. Anal Bioanal Chem. 2015 Sep;407(22):6815-25.; Wedemeyer H, et al. N Engl J Med. 2023 Jul 6;389(1):22-32.

# EON mediated NTCP editing demonstrates reduced clearance in bile acids challenge assay in NHP

**TUDCA elimination rate from plasma in NHP**

*(Exploratory study, early generation EON, n=5-7, 10mg/kg, 4 doses, SC, D51)*

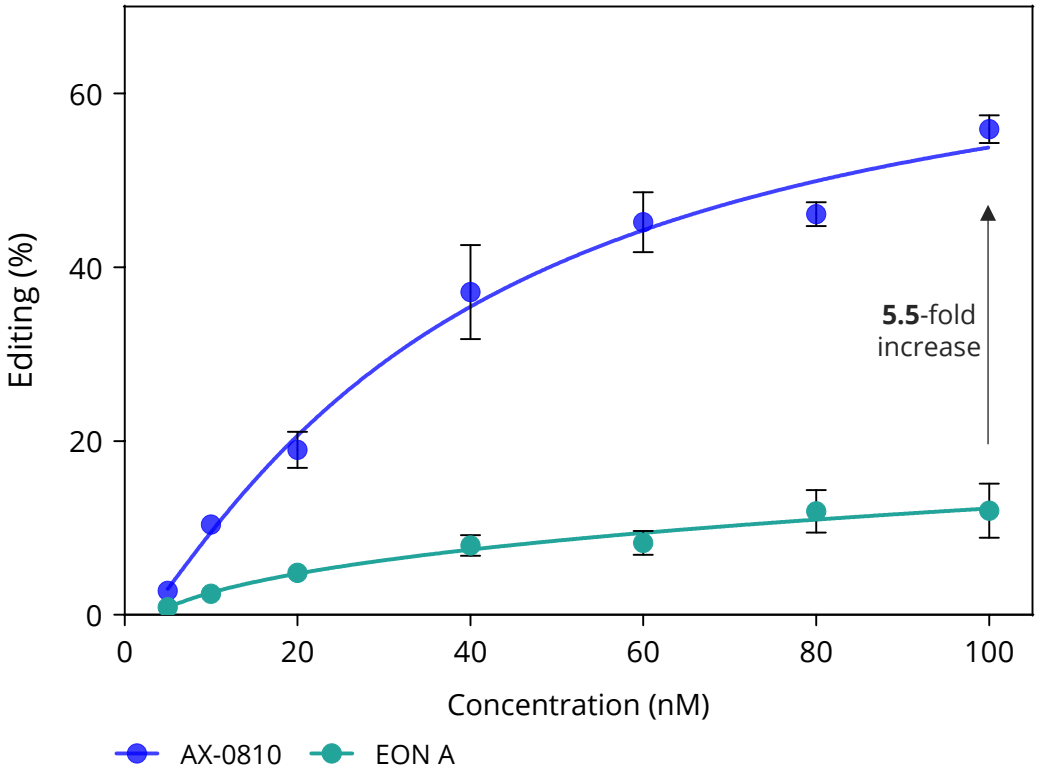



- TUDCA is a Tauro-conjugated bile acids specifically transported by NTCP from the plasma to the liver
- In an NHP experiment using administration of TUDCA following NTCP EON treatment, TUDCA plasma clearance into the liver was assessed
- Decrease in plasma clearance kinetics further confirm NTCP target engagement for EON treated NHP

# AX-0810 clinical candidate selected with enhanced potency and stability profile

**AX-0810 clinical candidate has an enhanced potency profile over EON A in PHH**

*Transfection, n=3, 72 hours, dPCR, mean±SEM*



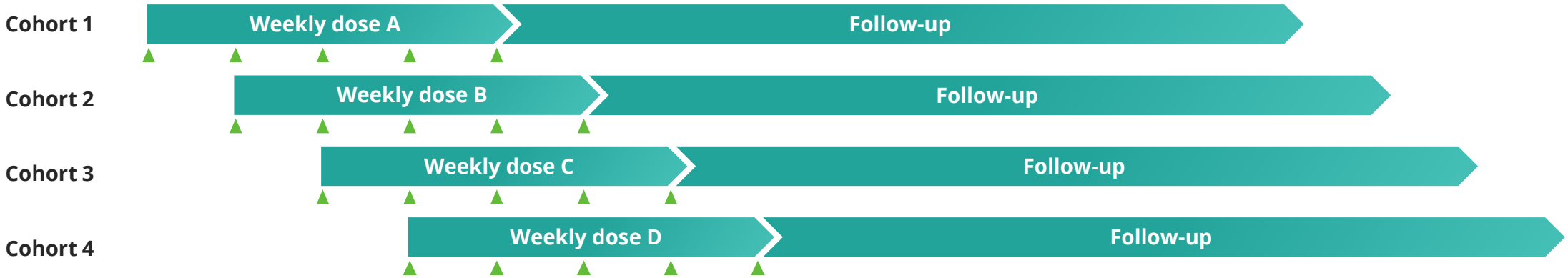

- AX-0810 clinical candidate is a GalNAc conjugated EON
- 5.5-fold increase in potency over early generation NTCP editing oligonucleotide
- Improved stability profile *in vitro*
- Confirmed class safety, with no hepatotoxicity or immunostimulatory score

# CTA enabling activities ongoing for AX-0810

| *In vitro* safety screening | Delivery method | GLP tox studies | Manufacturing | Regulatory |
|---|---|---|---|---|
| • AX-0810 clinical candidate passed in vitro screening for class toxicities<br>• Chemical modifications and Z-base derisked in genotoxicity tests | Preferential distribution of GalNAc conjugated EONs confirmed | • Dose ranges and margins established for GLP toxicity studies, ongoing studies in two species<br>• Bioanalytical methods to measure clinical candidates in plasma and tissue established | Scale-up of EON manufacturing process successfully completed, stability of formulated EON confirmed, and favorable shelf life achieved | Interactions with regulatory authorities ongoing |

# First in human trial of AX-0810 to establish target engagement

## Integrated single/multiple ascending dose study design




**Treatment**

AX-0810 GalNAc conjugated editing oligo-nucleotide

**Objectives**

- Confirm target engagement as measured by biomarkers
- Assess safety, tolerability, and PK of AX-0810

**Trial design**

- Combined single and multiple ascending dose
- ≥60 heathy volunteers, 4 weeks dosing phase followed by 12 safety weeks follow-up
- 5 weekly subcutaneous injections
- Baseline and placebo-controlled design
- Standardized conditions for assessment of bile acids at multiple timepoints
- DMC safety reviews before proceeding to next dose and dose escalation

**Key endpoints**

- Change in bile acids levels and profile in plasma and urine, liver biomarkers
- Circulating RNA as exploratory endpoint

**Top-line data in Q4 2025**

# Summary & next steps

*AX-0810 for cholestatic diseases*

## Modulating NTCP activity to reduce hepatic bile acids load is a promising target for hepatoprotection in cholestatic diseases

## Promising and consistent results reported to date in humanized mice and NHPs

- ✓ Meaningful impact on bile acid plasma level and bile acids profile build confidence for data readout in FIH clinical trial
- ✓ Axiomer NTCP EON impact on biomarkers in line with preclinical disease model and clinical data reported with NTCP inhibition
- ✓ Favorable safety profile observed
- ✓ AX-0810 GalNAc candidate with optimized potency and stability to enter clinic

## CTA submission in Q2 2025

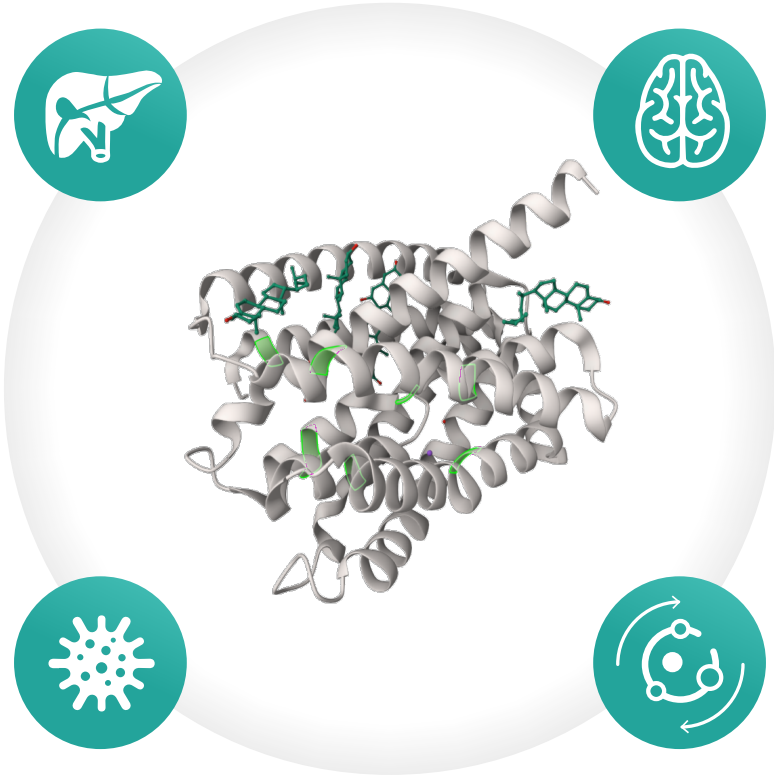
## Top-line data from FIH expected in Q4 2025

# NTCP and bile acids are involved in a variety of therapeutic areas

*Providing opportunity across multiple indications*

## Cholestatic diseases

- Primary Sclerosing Cholangitis (PSC)
- Biliary Atresia
- Primary Biliary Cholangitis (PBC)
- Alagille syndrome
- Dubin-Johnson Syndrome
- Progressive Familial Intrahepatic Cholestasis (PFIC)
- Drug-Induced Cholestasis
- Alcoholic Liver Disease
- Secondary Biliary Cirrhosis
- Rotor syndrome
- Neonatal cholestasis



## Neurological diseases

- Multiple Sclerosis
- Amyotrophic Lateral Sclerosis
- Neurological diseases
- Epilepsy
- Parkinson's Disease

## Infectious disease

- Parasitic Infections
- Sepsis-Associated Cholestasis
- Viral Hepatitis: Hepatitis A, B, C, D, E

## Metabolic diseases

- Hyperlipidemia
- Hypertension
- MASH
- Obesity
- Diabetes
- Lysosomal storage diseases
- Hyper-cholesterolemia
- ASCVD

# AX-2402 Program

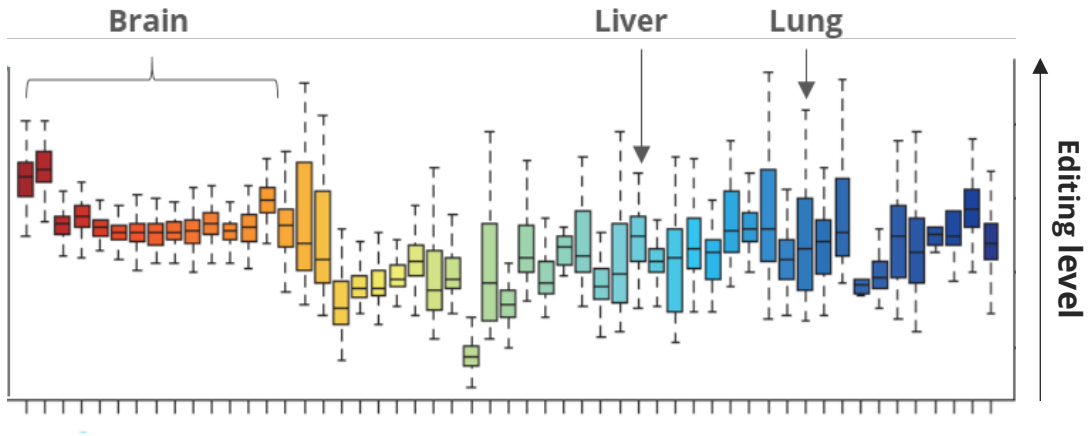
*Targeting MECP2 to restore protein functionality in Rett Syndrome, a severe neurodevelopmental disorder*

Presenters: Monica Coenraads, MBA and Gerard Platenburg

# CNS is a prime target organ for Axiomer RNA editing technology

- Numerous neurological disorders lack effective therapies, urge for new therapeutic approaches
- ADAR enzymes are highly expressed in the brain with very active editing capacity
- EONs have shown broad distribution, durability and were observed to have a favorable safety profile making them a well-suited approach for CNS indications

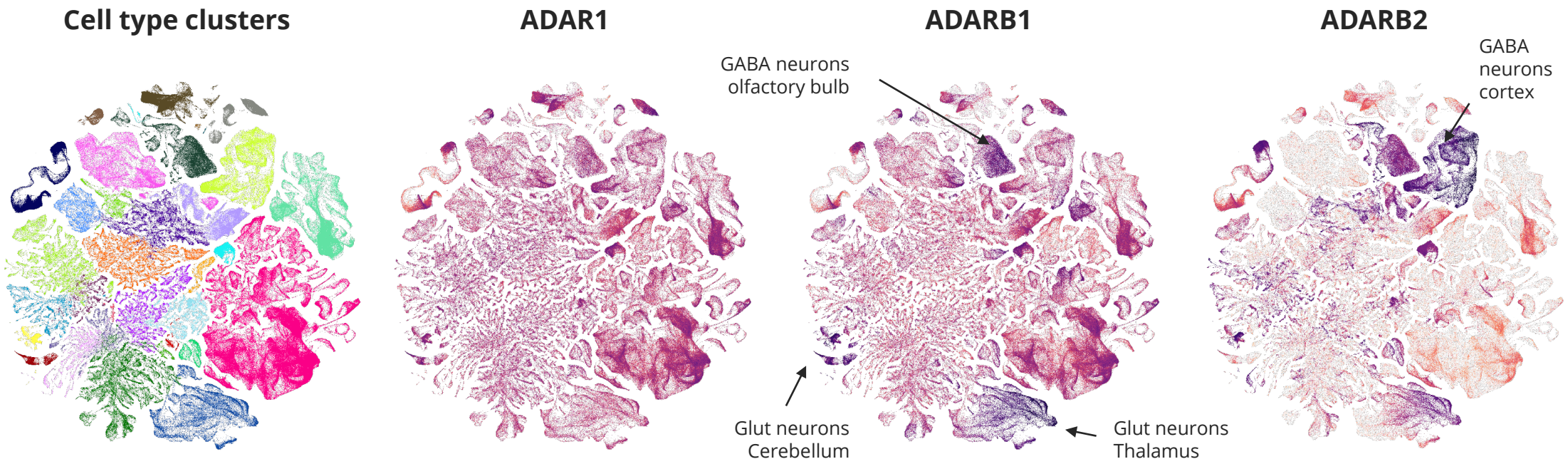
**ADAR mediated A-to-I editing in human tissues[1]**




[1]Figure adapted from Tan et al. Nature. 2017 Oct 11;550(7675):249-254

# Robust ADAR expression across cell type and regions in mouse brain

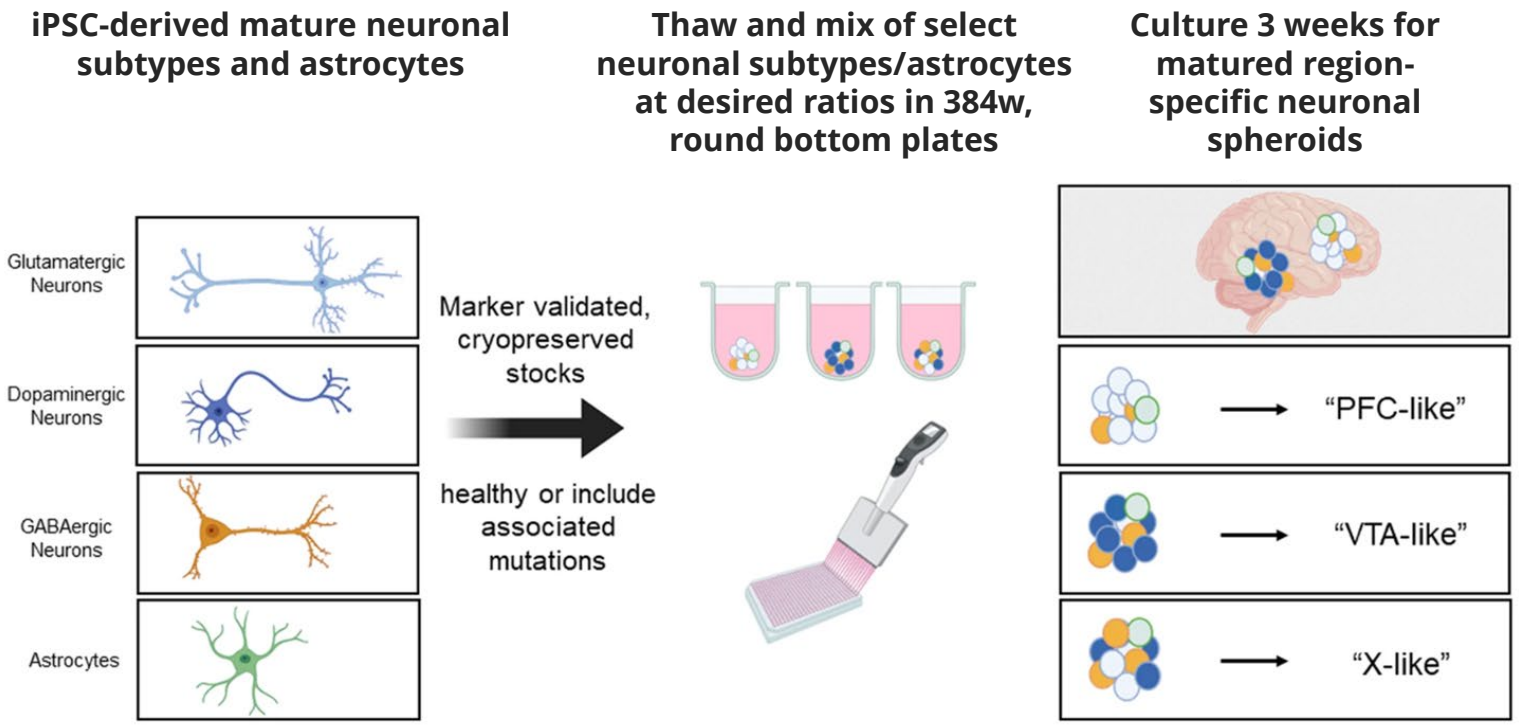
*Cell type specific expression of Adarb1 and Adarb2 genes*




High expression of Adar genes in different cell type and regions in the mouse brain

Whole Mouse Brain Transcriptomic Cell Type Atlas – Allen brain atlas

# Predictive CNS models to inform development of RNA editing

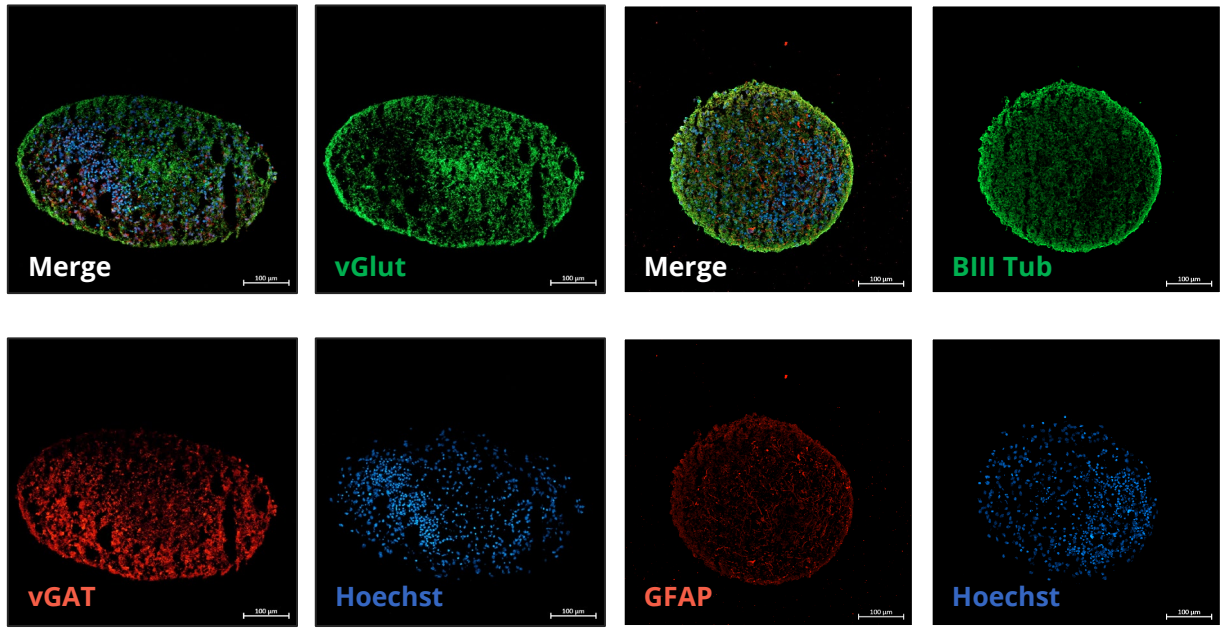



- Spheroids are 3D cultures that model specific brain regions depending on the mix of hIPSC-derived neuronal subpopulations used
- They can give rise to pre-frontal cortex-like (PFC`) or ventral tegmental area (VTA)-like 3D structures
- Uniformly-shaped PFC, form within 24-48 hours with size yield of ~400 µm

Development of reproducible, region-specific neural stem cell (NSC)-derived spheroids addresses limitations of 3D iPSC-derived organoids, offering a robust and predictive tool for accelerating drug discovery in neurodegenerative diseases, substance abuse, and pain management.

# Highly efficient RNA editing in brain organoid recapitulating human cortex

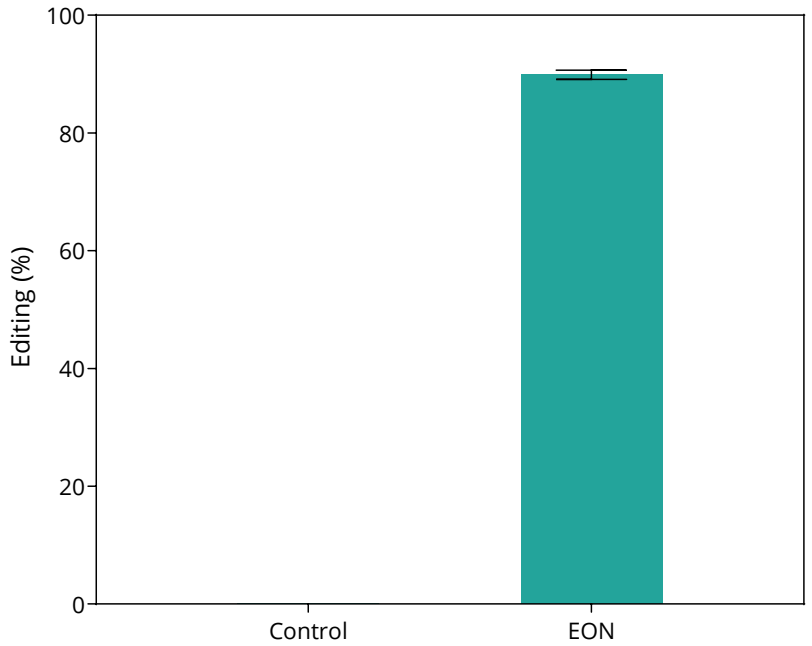
*Reaching 90% editing in neurospheroids*




PFC-like spheroids are composed by 90% neurons and 10% astrocytes and exhibit a 70:30 ratio of excitatory (Glutamatergic) and inhibitory (GABAergic) neurons recapitulating the cellular composition of the human cortex

**RNA editing of APP in human PFC-like spheroids**

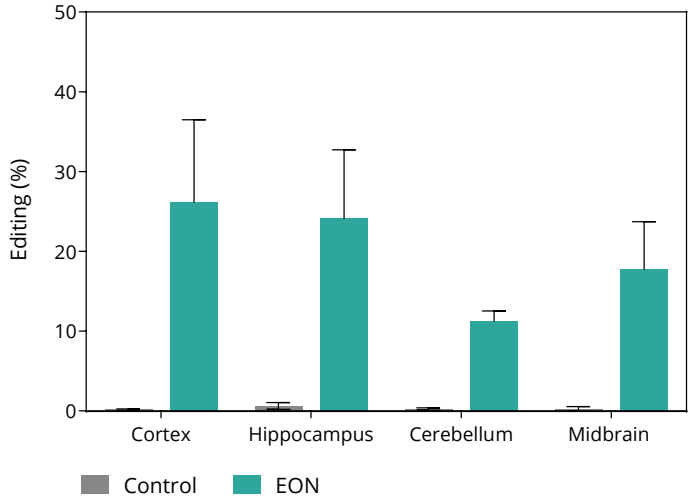
*Transfection, 5 µM, single dose, n=3, 7 days, mean, SD, ddPCR*



# Consistent CNS editing demonstrated across species

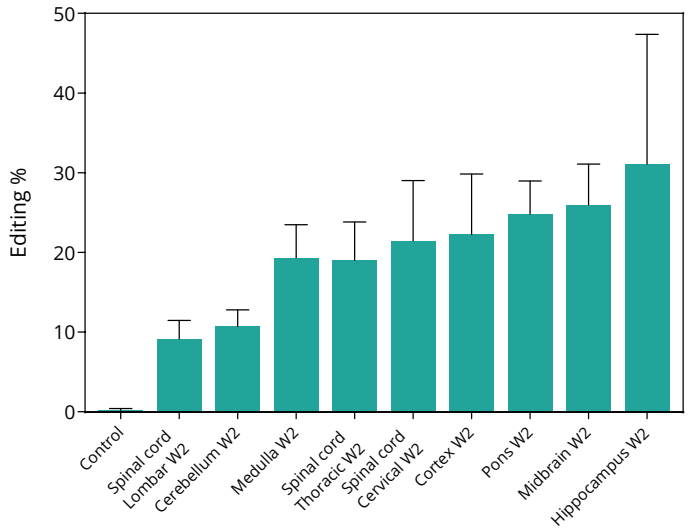
## MICE *in vivo*

*ICV, 250µg, undisclosed target, single dose, n=6, 4 weeks, ddPCR, mean, SD*



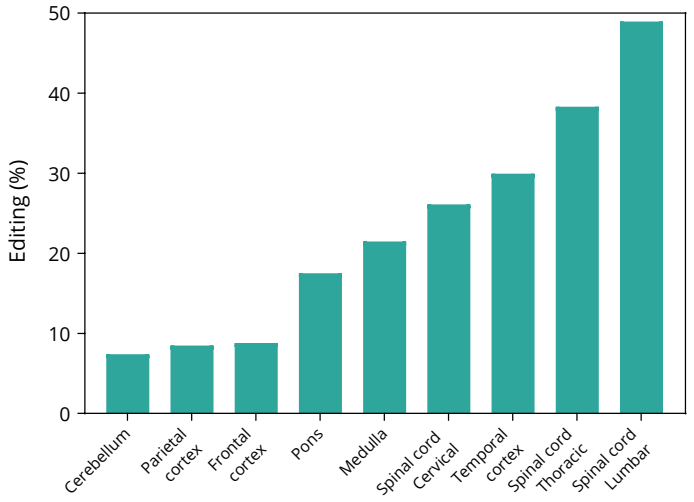

## RAT *in vivo*

*ICV, 500µg, APP, single dose, n=5, 2 weeks, ddPCR, mean, SD*




## NHP *in vivo*

*IT administration, undisclosed target 12mg, single dose, n=3*, 7 days, ddPCR*




- Up to 40% editing *in vivo* leading to 26-fold change in protein function recovery in brain tissues of interest at 4 weeks with a single dose in mice model
- In rat, Axiomer EONs demonstrated up to 50% editing *in vivo* with sustained editing between W2 and W4 after single dose
- Up to 30% RNA editing reported in brain and approx. 50% in spinal cord in NHP in vivo

* Data of 2 NHPs not analyzable due to human error during injection procedure.

# Axiomer™ holds strong potential to make a meaningful impact to CNS diseases

## Strong RNA Editing Performance

- Robust RNA editing in critical CNS regions validating the efficiency of Axiomer platform in CNS indications

## Broad Applicability Across CNS Regions

- RNA editing was successfully achieved in multiple regions of the nervous system, indicating the platform's broad applicability across different CNS regions

## Consistent and Durable Results with Well Understood Safety Profile

- Consistent RNA editing across species, with durable effects observed
- EONs have been observed to have a favorable safety profile in CNS

# AX-2402 RNA editing therapy targeting MECP2 for Rett Syndrome

RETT SYNDROME RESEARCH TRUST

- Rett Syndrome is a **devastating and progressive neurodevelopmental disorder** caused by variants in the transcription factor Methyl CpG binding protein 2 (*MECP2*). There is a **high unmet need for a disease modifying therapy**.
- Nonsense variants lead to **severe phenotypes.** They represent more than one third **of Rett Syndrome** cases and are projected to affect **20,000 individuals** in US and EU.[1,2]
- Rett Syndrome is **not a neurodegenerative disorder** and restoring levels of the MECP2 protein has shown to **reverse symptoms** in mice.[3]
- Axiomer has the potential to **restore the precise level of MECP2 protein regulatory function,** which is lacking in Rett Syndrome, and become a disease modifying therapy.
- Rett Syndrome Research Trust partnership includes $9.1 M in funding; collaboration established in January 2024, expanded in December 2024


[1]Krishnaraj R, et al. Hum Mutat. 2017 Aug;38(8):922-93; [2]RSRT 2023 conference; [3]Guy J, et al. Science. 2007 Feb 23;315(5815):1143-7.

# Monica Coenraads, MBA

*Founder, Chief Executive Officer at Rett Syndrome Research Trust*

- Monica Coenraads' involvement with Rett syndrome began the day her then-two-year-old daughter was diagnosed with the disorder. A year later, in 1999, she co-founded the Rett Syndrome Research Foundation (RSRF) and held the position of scientific director during the eight years of the Foundation's drive to stimulate scientific interest and research in Rett syndrome, culminating with the groundbreaking work in 2007 which demonstrated the first global reversal of symptoms in preclinical models of the disorder. Monica launched the Rett Syndrome Research Trust in late 2008 to pursue the next steps from that milestone.
- As chief executive officer she oversees all aspects of the organization, including day-to-day operations, strategic direction, fundraising, and communications. Together with her colleagues and with input from advisors and the scientific community at large, Monica sets and executes RSRT's research agenda.
- Under Monica's leadership at RSRF and RSRT, $117 million has been raised for Rett syndrome.

# AX-2402 RNA editing therapy targeting MECP2 for Rett Syndrome

RETT SYNDROME RESEARCH TRUST

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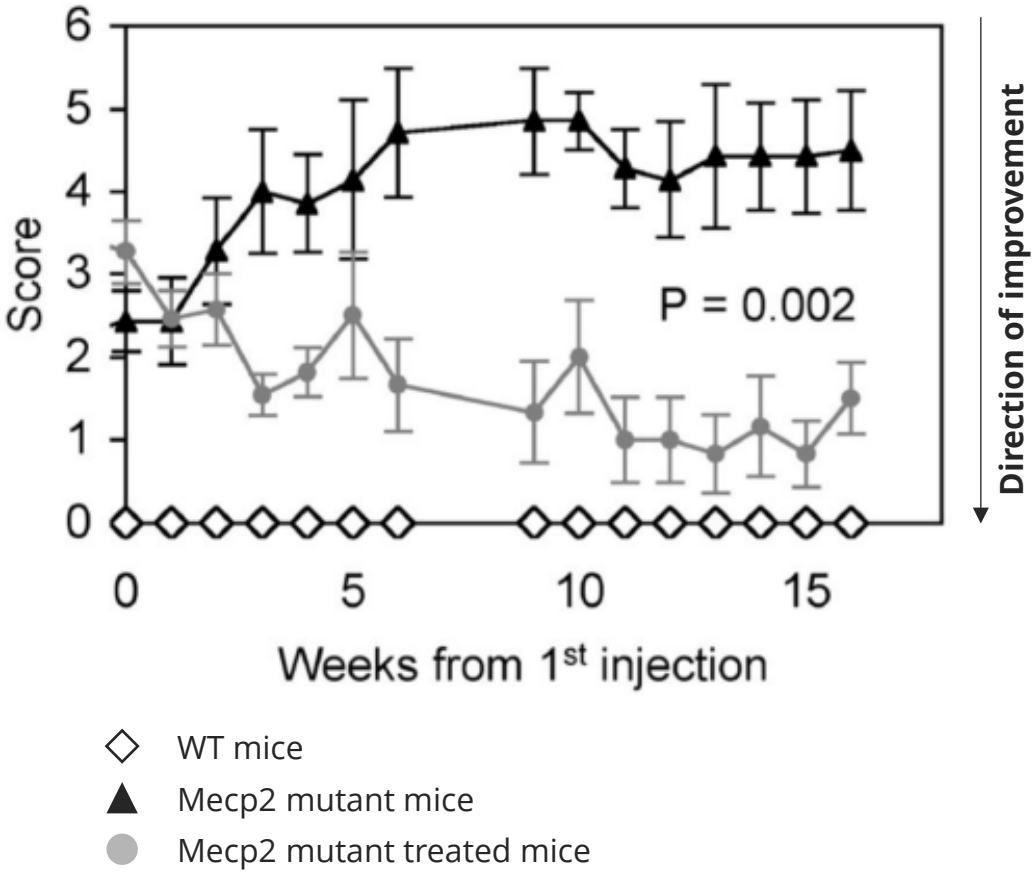
# MECP2 gene is frequently mutated in Rett syndrome (RTT)

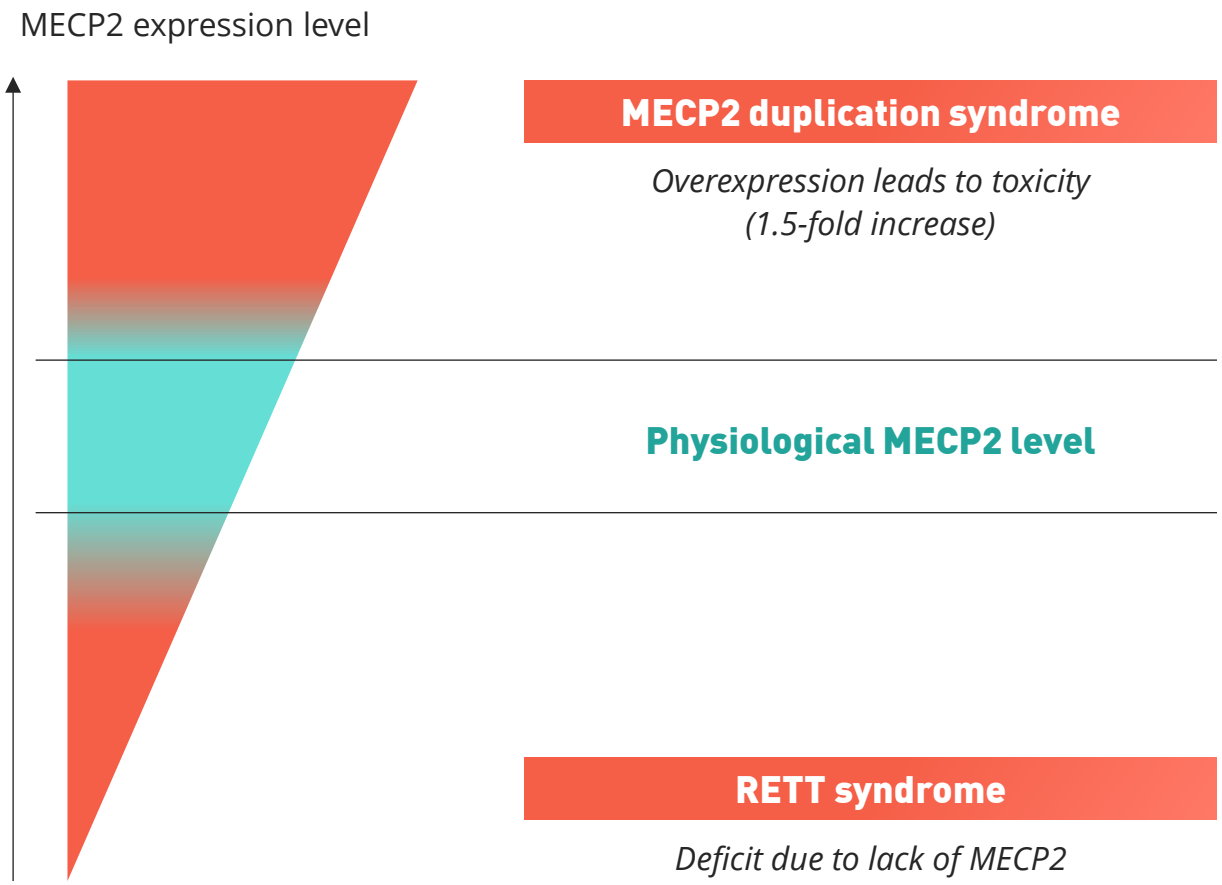
- MECP2 gene, encoding methyl-CpG binding protein 2 (MeCP2):
  - Master epigenetic modulator of gene expression and plays a vital role in neuronal maturation and function
  - Mutations lead to misfolded, truncated or absent protein and loss of function
  - This loss of MECP2 regulating function leads to Rett syndrome and 35% of point mutations cause a premature termination codon (PTC)
- In 2007, Adrian Bird's lab demonstrated that Rett syndrome symptoms are reversible in mice[1]




[1]Guy J, et al. Science. 2007 Feb 23;315(5815):1143-7. Figure adapted from Guy J, et al. Science. 2007 Feb 23;315(5815):1143-7.

# MECP2 expression level tightly regulated in neurons

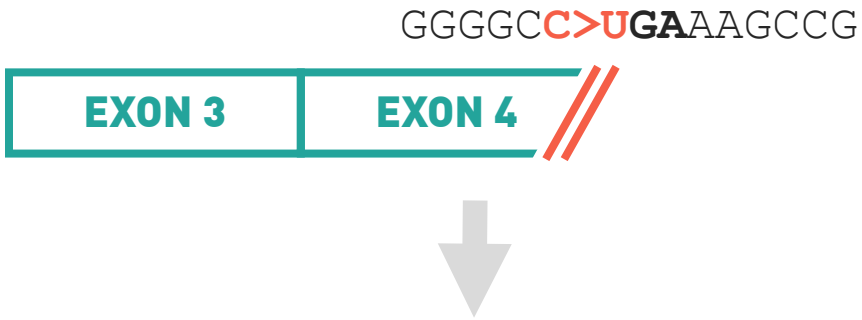
*Axiomer is a well-suited approach to restore physiological levels of MECP2*




- Axiomer approach makes use of ADAR endogenous system to restore physiological levels of functional MECP2
- Axiomer avoid the risk of expressing unsafe levels of MECP2, potentially leading to MECP2 duplication syndrome

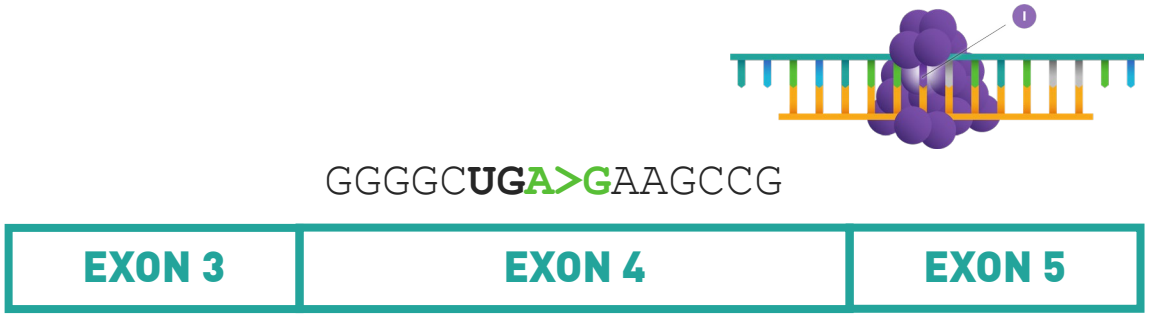
# Axiomer™ has the potential to restore physiological levels of functional MECP2

*AX-2402 correcting MECP2 R270X into WT-like R270W*




**RETT syndrome**

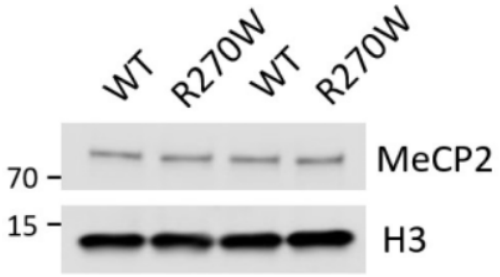
Postnatal microcephaly, stereotypic hand movements, ataxia, abnormal breathing, and growth retardation, social withdrawal, loss of speech, seizures

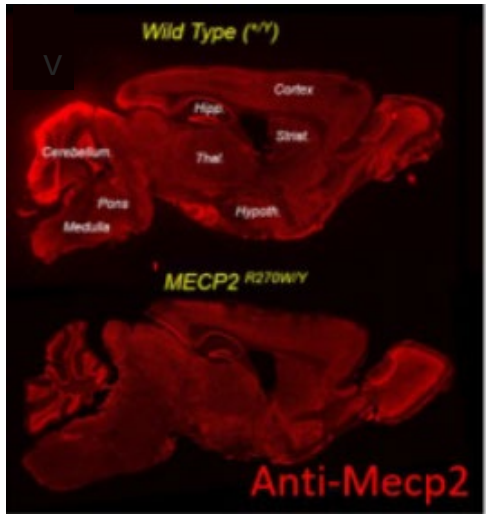



**WT like phenotype**

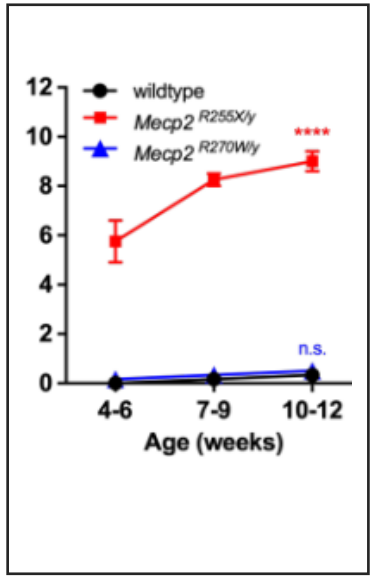
- MeCP2 protein restoration/recovery
- MeCP2 R270W (Arg > Trp) mouse model indistinguishable from wild type mice

# R270W variant demonstrates wild-type like profile

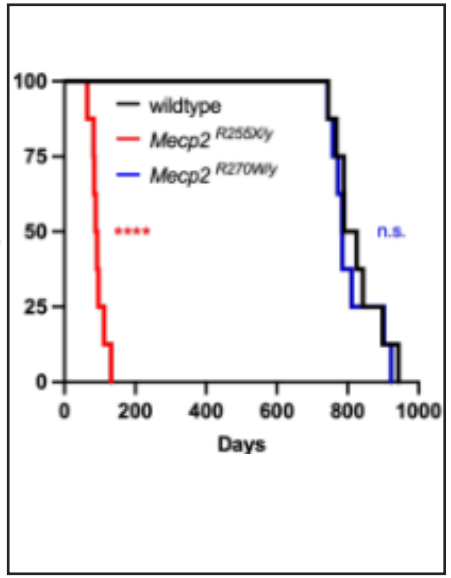


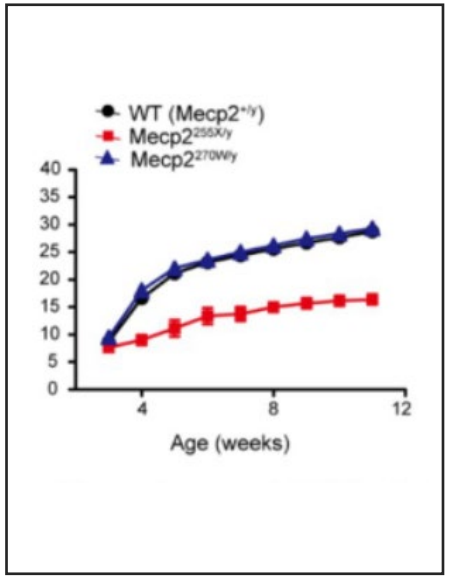

**Severity score (0-12)**



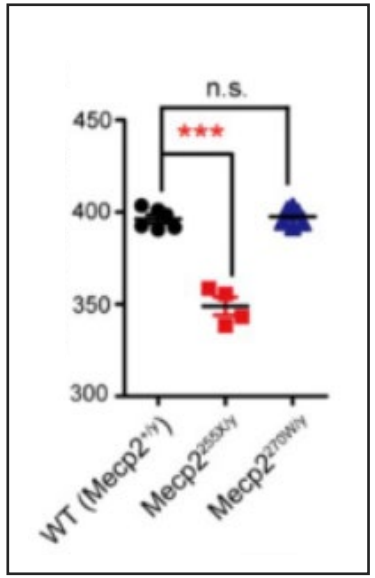

**Probability of survival**




**Body weight (g)**




**Brain weight (mg)**

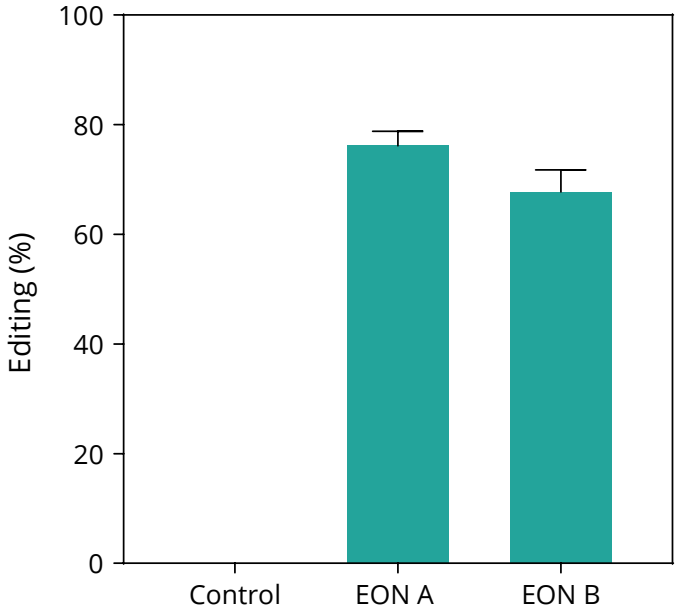



AX-2402 can restore physiological levels of functional MECP2 potentially reverting Rett syndrome into a WT like phenotype[1]

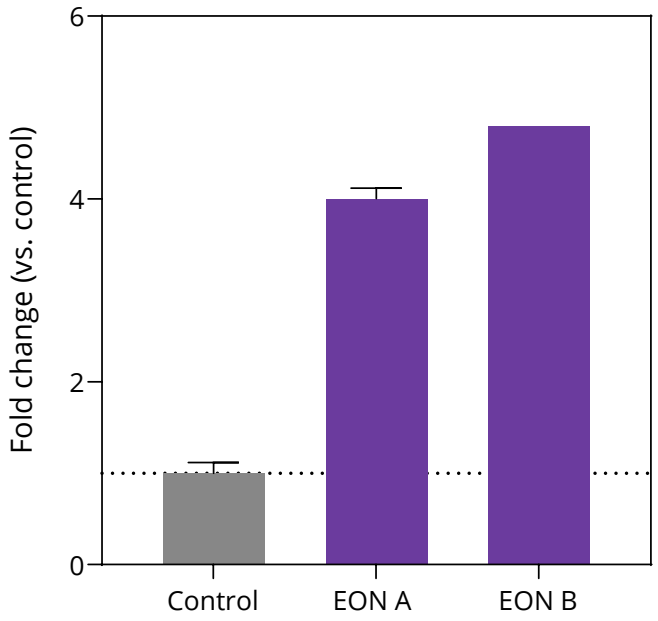
[1]Colvin, S. (2023) thesis. Massachusetts Institute of Technology. Figures adapted from: Colvin, S. (2023) thesis. Massachusetts Institute of Technology

# EON mediated editing in patient's cells increases mRNA levels and restores protein expression

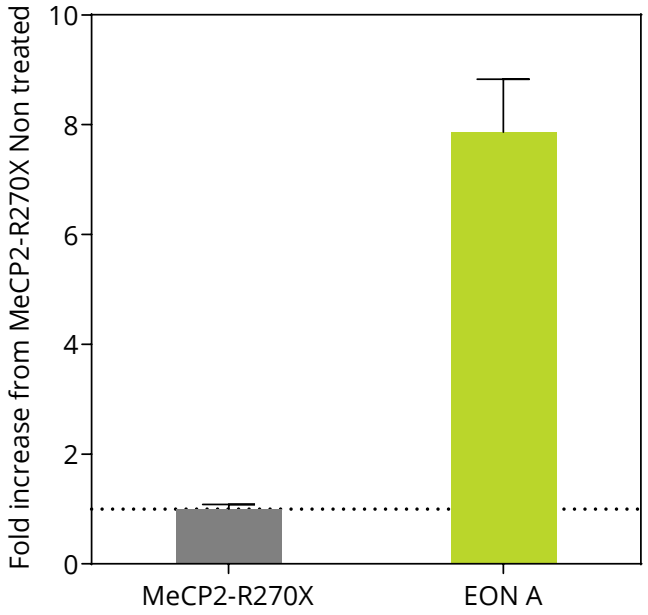
*PTC recoding leading to absent NMD mediated RNA degradation*




Up to 80 % editing of R270X MECP2 in patient fibroblasts




Increased MECP2 RNA levels due to PTC recoding and NMD inhibition

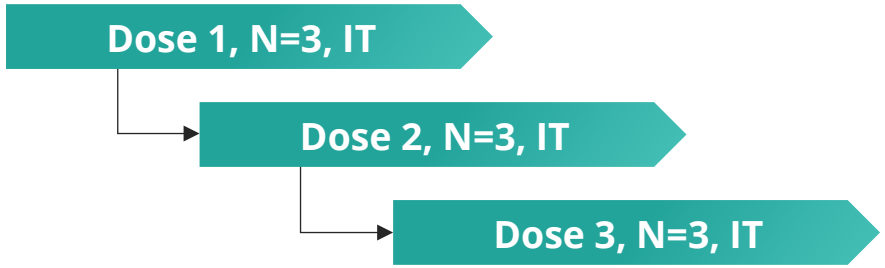



Increased R270W MECP2 protein levels

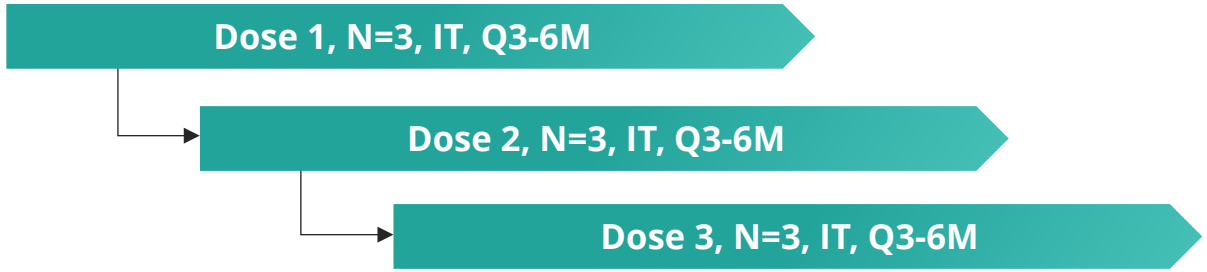
EON, Editing oligonucleotide; NT, Non-treated; TF, transfection, Conditions panel on the left and middle: 100 nM EON, transfection, 48h, N=2, mean±SEM. Conditions panel on the right: MeCP2-R270X-NanoLuc activity; 100 nM EON, transfection, 48h, N=8, mean±SEM.

# Preliminary clinical trial design

**Single dose**




**Repeated dose**




- Preliminary Phase 1/2 SAD & MAD design
- Up to 18 subjects with the R270X mutation
- Primary objective: safety, tolerability and pharmacokinetics
- Secondary objectives: target engagement and biomarkers
- Financially supported by $8.1M funding provided by Rett syndrome Research Trust
- **Clinical candidate selection in 2025**
- **Top-line data expected in 2026**

# AX-1412 Program

*Targeting B4GALT1 to reduce the risk of cardiovascular diseases*

Presenter: Gerard Platenburg

# AX-1412 RNA editing therapy targeting B4GALT1 for cardiovascular diseases

**Leading causes of death in the world**
**~18 million people** die from CVDs every year (**32% of all global deaths**) Despite therapies, the unmet medical need remains.

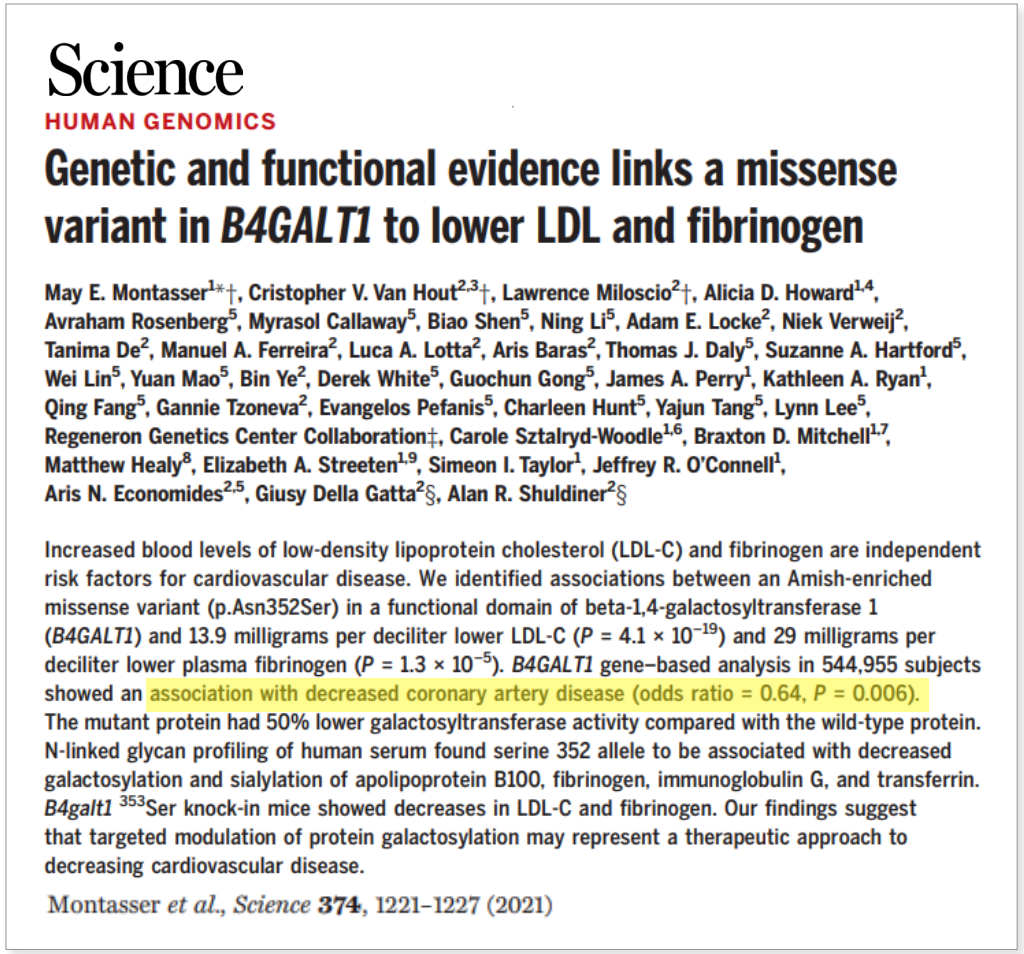
AX-1412 is designed to provide people with a protective genetic variant of B4GALT1 that is associated with **36%[1] reduction in the risk of cardiovascular disease**.

AX-1412 may become a **stand-alone cardiovascular therapy** that may also work **synergistically with standard of care** to further reduce risk of CVDs.


[1]Montasser ME, et al. Science. 2021 Dec 3;374(6572):1221-1227

# B4GALT1 p.Asn352Ser variant reduces CVD risk

- It is described that people who carry missense variants like the p.Ans352Ser in B4GALT1, **have 36% lower chance of the development of coronary artery disease.**[1] This variant is known as the “old Amish order variant”
- This variant reduces CVD risk through 2 independent risk factors, fibrinogen and LDL-C, through independent pathways from PCSK9
- This protective variant is a A-to-G variant, on that can be introduced by Axiomer mediated ADAR editing
- B4GALT1 is not suitable for knockdown technologies, as leads to semi-lethality and severe development abnormalities in mouse studies



Science

HUMAN GENOMICS

## Genetic and functional evidence links a missense variant in *B4GALT1* to lower LDL and fibrinogen

May E. Montasser[1*†], Cristopher V. Van Hout[2,3†], Lawrence Miloscio[2†], Alicia D. Howard[1,4], Avraham Rosenberg[5], Myrasol Callaway[5], Biao Shen[5], Ning Li[5], Adam E. Locke[2], Niek Verweij[2], Tanima De[2], Manuel A. Ferreira[2], Luca A. Lotta[2], Aris Baras[2], Thomas J. Daly[5], Suzanne A. Hartford[5], Wei Lin[5], Yuan Mao[5], Bin Ye[2], Derek White[5], Guochun Gong[5], James A. Perry[1], Kathleen A. Ryan[1], Qing Fang[5], Gannie Tzoneva[2], Evangelos Pefanis[5], Charleen Hunt[5], Yajun Tang[5], Lynn Lee[5], Regeneron Genetics Center Collaboration‡, Carole Sztalryd-Woodle[1,6], Braxton D. Mitchell[1,7], Matthew Healy[8], Elizabeth A. Streeten[1,9], Simeon I. Taylor[1], Jeffrey R. O'Connell[1], Aris N. Economides[2,5], Giusy Della Gatta[2]§, Alan R. Shuldiner[2]§

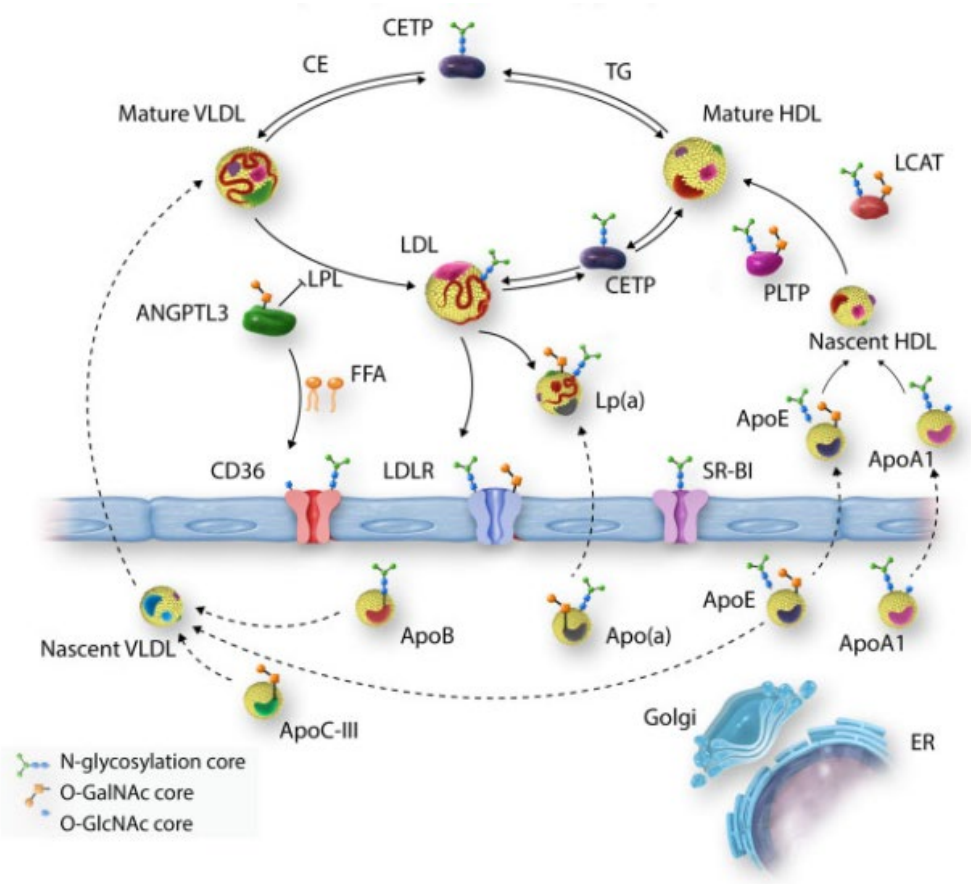
Increased blood levels of low-density lipoprotein cholesterol (LDL-C) and fibrinogen are independent risk factors for cardiovascular disease. We identified associations between an Amish-enriched missense variant (p.Asn352Ser) in a functional domain of beta-1,4-galactosyltransferase 1 (*B4GALT1*) and 13.9 milligrams per deciliter lower LDL-C ($P = 4.1 \times 10^{-19}$) and 29 milligrams per deciliter lower plasma fibrinogen ($P = 1.3 \times 10^{-5}$). *B4GALT1* gene–based analysis in 544,955 subjects showed an association with decreased coronary artery disease (odds ratio = 0.64, $P = 0.006$). The mutant protein had 50% lower galactosyltransferase activity compared with the wild-type protein. N-linked glycan profiling of human serum found serine 352 allele to be associated with decreased galactosylation and sialylation of apolipoprotein B100, fibrinogen, immunoglobulin G, and transferrin. *B4galt1* $^{353}$Ser knock-in mice showed decreases in LDL-C and fibrinogen. Our findings suggest that targeted modulation of protein galactosylation may represent a therapeutic approach to decreasing cardiovascular disease.

Montasser *et al.*, *Science* **374**, 1221–1227 (2021)

[1]Montasser ME, et al. Science. 2021 Dec 3;374(6572):1221-1227

# Glycosylation is a key process in lipid metabolism

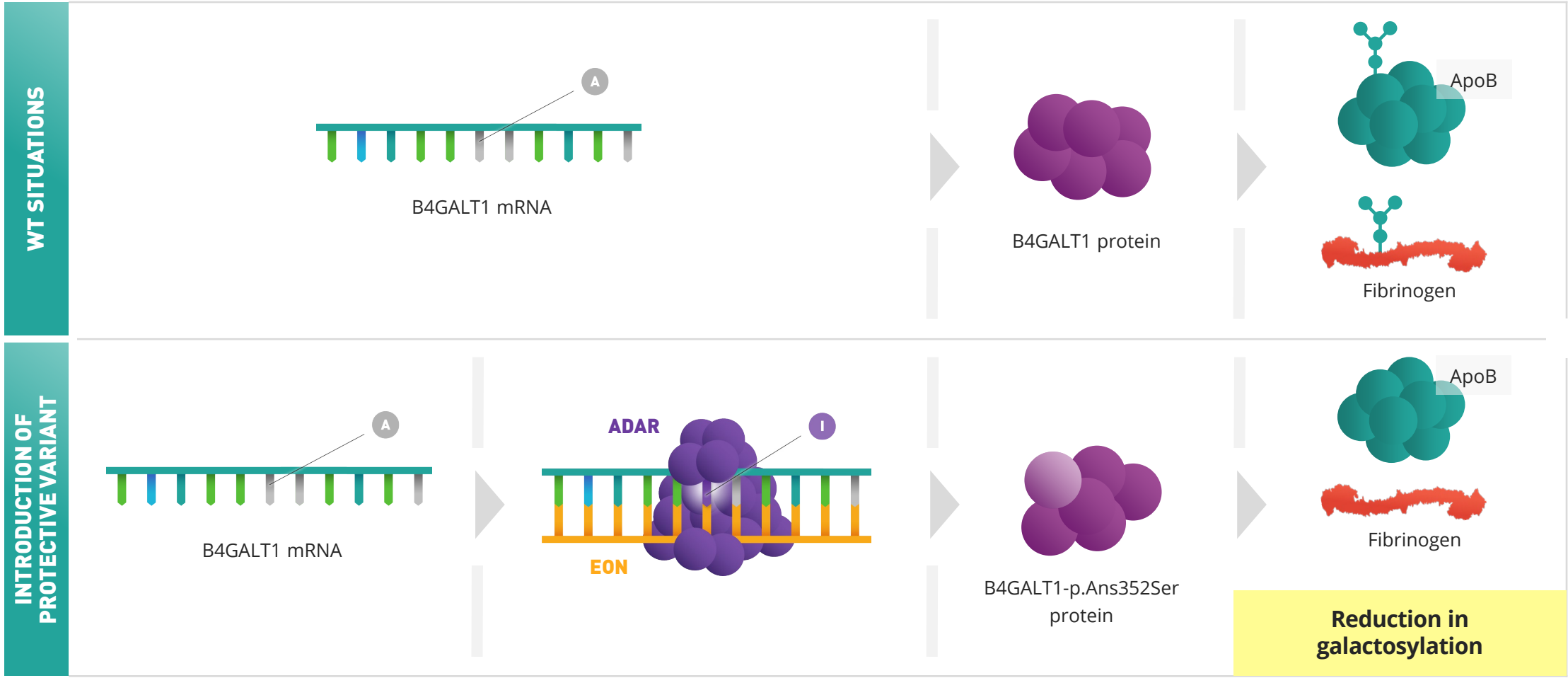
**Physiological lipoprotein glycosylation[1]**




- Glycosylation stabilizes the folding and conformation of apolipoproteins (e.g., ApoB, ApoE), ensuring proper assembly and secretion of lipoproteins such as LDL and HDL.
- Glycosylation of receptors like LDLR is critical for their membrane localization and ligand binding, enabling efficient lipoprotein clearance from the bloodstream.
- Aberrant glycosylation can lead to dysfunctional lipoproteins, a key driver of atherosclerosis.

[1]Pirillo A, et al. Cardiovasc Res. 2021 Mar 21;117(4):1033-1045.

# B4GALT1 p.Asn352Ser variant to reduce galactosylation of CVD risk factors

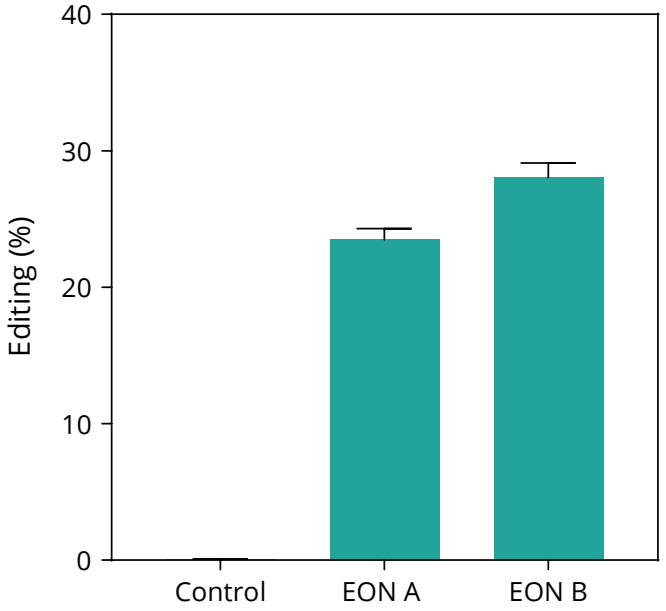


# EON-mediated editing of B4GALT1 leads to reduced glycosylation activity

*In line with natural population*

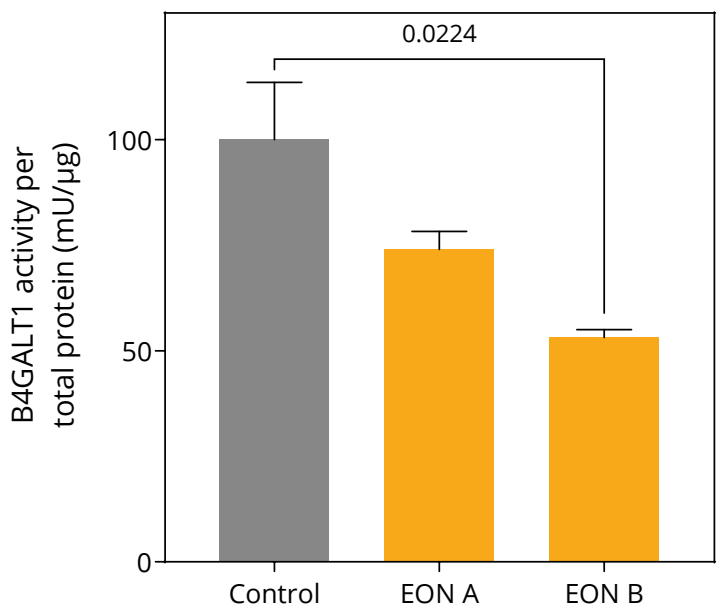
**EON mediated editing of B4GALT1 in PHH**

*5µM transfection, dPCR, mean, SD, n=3*



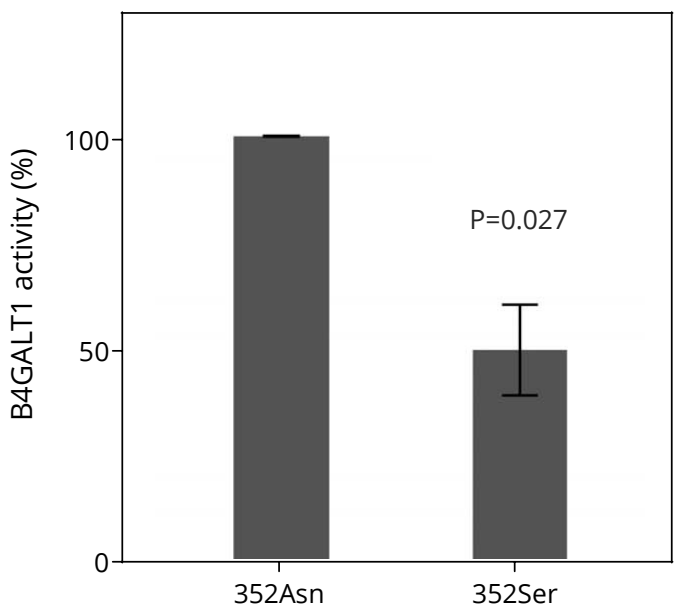

**B4GALT1 activity following editing**

*PMH, cell lysate, transfection 5µM, 4 days, mean, SD, ANOVA*




**B4GALT1 activity reported in Montasser et al.[1]**

*COS-7 cells, cell lysate, transfection, n=4, mean, SEM*

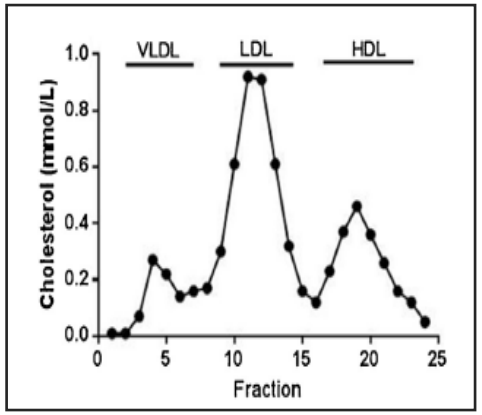



[1]Montasser ME, et al. Science. 2021 Dec 3;374(6572):1221-1227. Percentage of 352Asn B4GALT1 galactosylation activity of 352Asn B4GALT1 and 352Ser B4GALT1 immunoprecipitated proteins

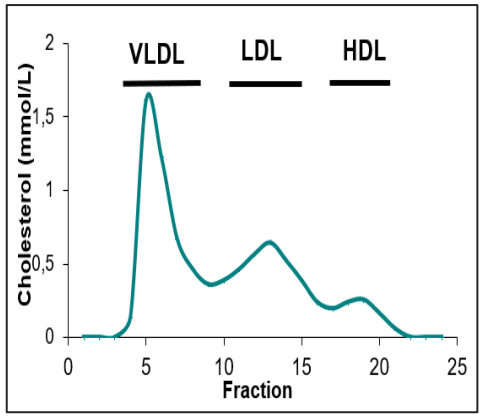
# E3L.CETP mice disease model is industry standard for assessing CVD therapeutics

- CETP facilitates the transfer of cholesteryl esters from HDL to VLDL and LDL, a key process in human lipid metabolism that is absent in most rodent models.
- These mice, fed a high-fat high-cholesterol diet (HFCD), exhibit a biphasic dyslipidemic response, closely mimicking plasma lipid changes in humans
- The presence of CETP in this model makes it uniquely suited to study dyslipidemia and cholesterol metabolism, especially in relation to B4GALT1, which is involved in glycosylation processes affecting lipid metabolism.
- In humans, most circulating lipids are confined to VLDL/LDL particles

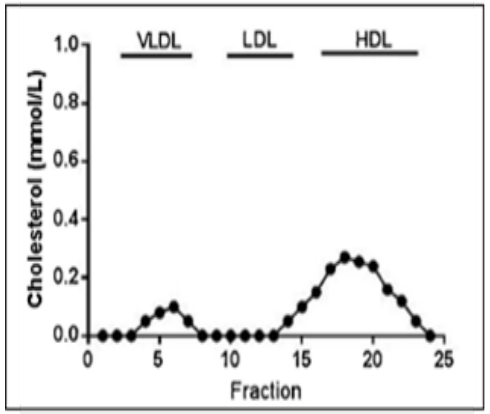
**Human**




**E3L.CETP mice disease model**




**Wildtype healthy mice**

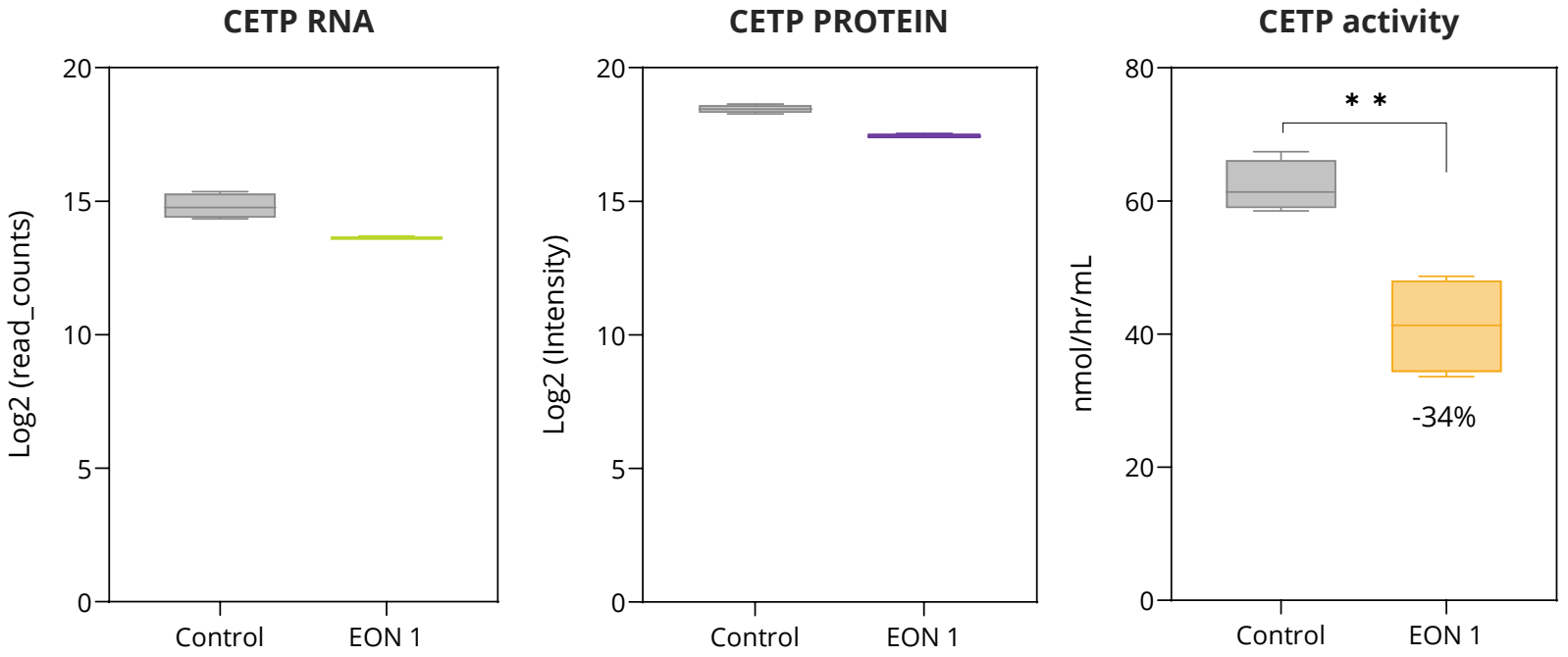


# B4GALT1 editing impacts activity of key proteins involved in lipid metabolism

*Minimal changes in transcript and protein levels associated with decrease in CETP activity in vivo*

**CETP RNA, protein and activity following EON B treatment**

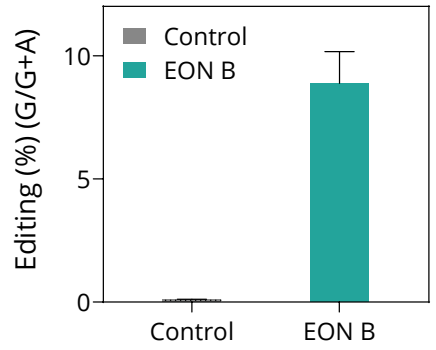
*E3L.CETP mice, LNP formulation, 2mg/kg, Q1W, D31, RNAseq, Roar, mean, max-min n=3, T-test*

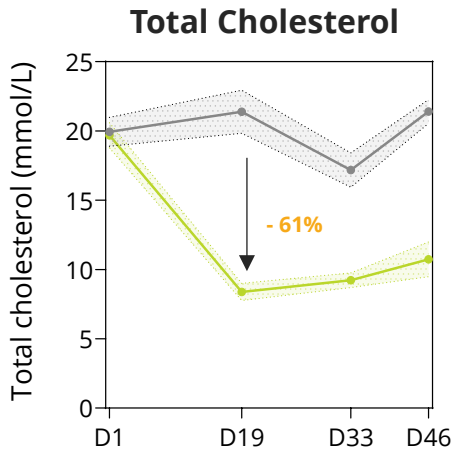


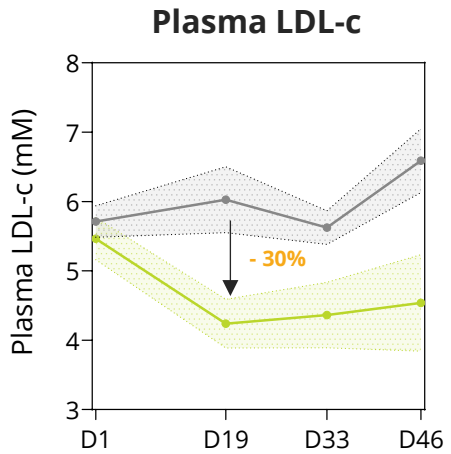

Reduced CETP activity in the absence of changes at the transcriptomic or proteomic levels highlights the impact of EON on glycosylation rather than on expression levels

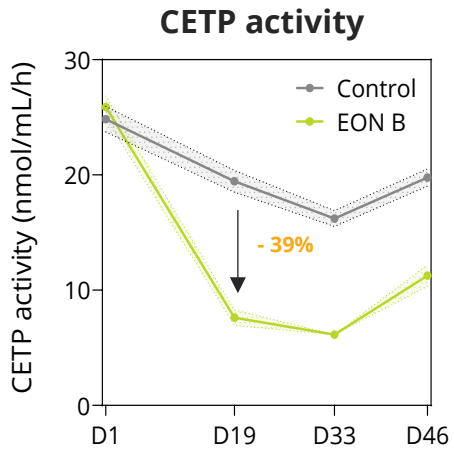
# EON-mediated editing of B4GALT1 leads to meaningful effect on key biomakers in E3L.CETP Mice

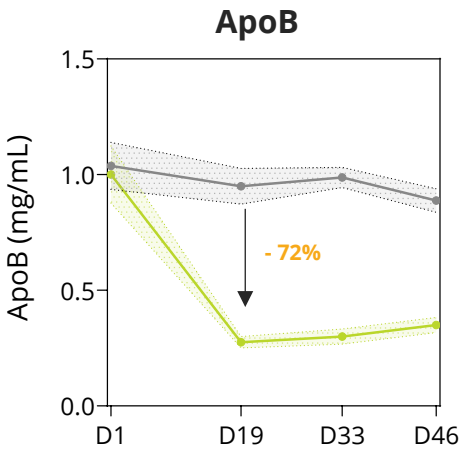
**B4GALT1 editing and biomarkers in E3L.CETP mice** *(N=10, 2mg/kg, LNP formulation, IV Q1W, D46, ddPCR)*

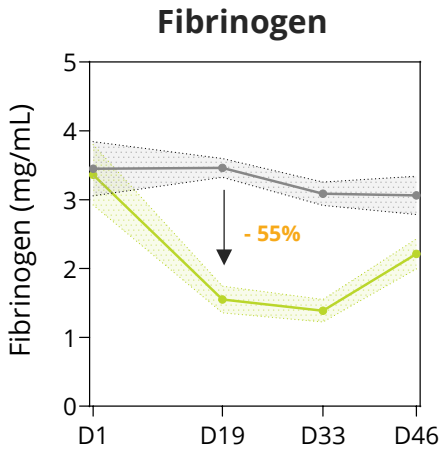


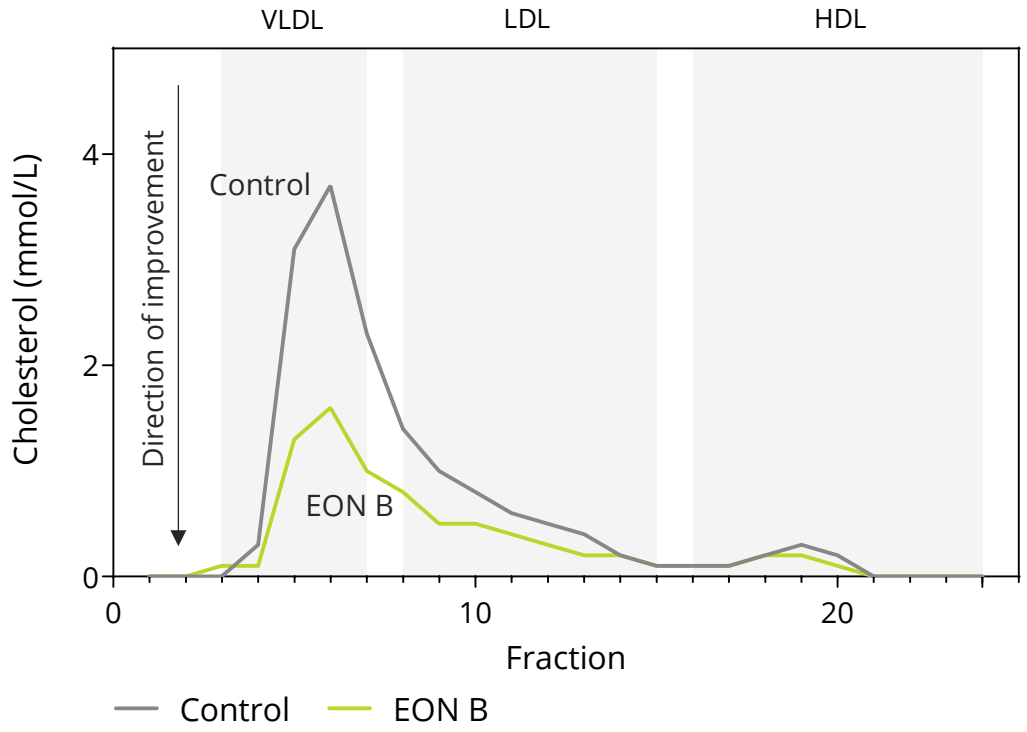

Following treatment with EON B, a marked reduction in total cholesterol, ApoB, and LDL-c by observed already at Day 19 confirms our approach to address cardiovascular diseases

# B4GALT1 EON leads to a positive shift in lipoprotein profiles

*Specifically targeting atherogenic lipoproteins*

**Impact on lipoprotein profile following editing of B4GALT1 in E3L.CETP mice**

*(N=10, 2mg/kg, LNP formulation, IV Q1W, D46)*




- Treatment with EON B significantly decreases VLDL and LDL cholesterol compared to control
- These lipoproteins are associated with increased cardiovascular risk due to their role in atherosclerotic plaque formation
- HDL cholesterol which supports reverse cholesterol transport and is associated with reduced cardiovascular risk, remains unchanged

# Summary & next steps

*AX-1412 for CVD*

## EON-mediated RNA editing of B4GALT1 leads to the required reduction in galactosylation

*reflecting the human genetics observed effect*

## LNP-delivered EON editing B4GALT1 leads to editing and meaningful changes

*in biomarker effect on LDLC, CEPT, cholesterol and fibrinogen in an industry-standard in vivo disease model*

## Further optimization of a GalNAc delivered EON ongoing

*to achieve a TPP desirable for CVD*

## Expected to provide an update on the optimization efforts in mid 2025

# AX-2911 Program

*Targeting PNPLA3 to address unmet medical needs in MASH*

Presenter: Gerard Platenburg

# AX-2911 RNA-editing therapy to address Metabolic dysfunction associated steatohepatitis (MASH)

MASH and subsequent stages of liver disease **are very prevalent and still on the rise worldwide**. MASH individuals have a high unmet medical needs due to the **progressive** nature of the disease (cirrhosis and hepatocellular carcinoma) and **limited therapeutic options** available[1]

PNPLA3 (patatin-like phospholipase domain-containing 3) I148M is a variant **commonly reported** in the MASH population worldwide (20-60% of the patients) and is known as **associated risk factor**.[2,3] Approximately 8 million individuals in US and EU are homozygous for the 148M variant.

Axiomer EONs have the potential to change the Methionine into a Valine bringing the **PNPLA3 protein back to a WT-like functional conformation**.

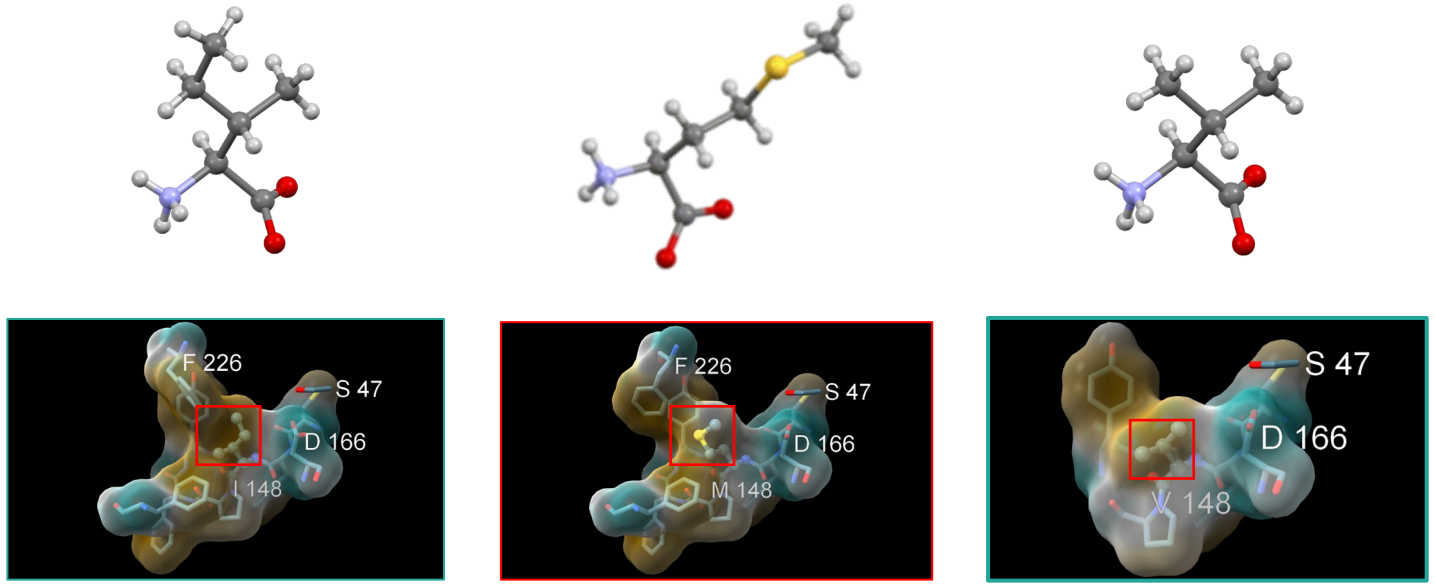

[1]Sandireddy R, et al. Front Cell Dev Biol. 2024 Jul 16;12:1433857; [2]Romeo S, et al. Nat Genet. 2008 Dec;40(12):1461-5; [3]Salari N, et al. BMC Endocr Disord. 2021 Jun 19;21(1):125.

# Axiomer™ creates a PNPLA3 protein with WT-like functionality

**PNPLA3 148I (WT)**
*Isoleucine (ATC)*

**PNPLA3 148M (Mutant)**
*Methionine (**A**TG)*

**PNPLA3 148V (Axiomer *de-novo* variant)**
*Valine (**G**TG)*




*F226 not included here*

Electrostatic potential

min max

Hydrophobicity

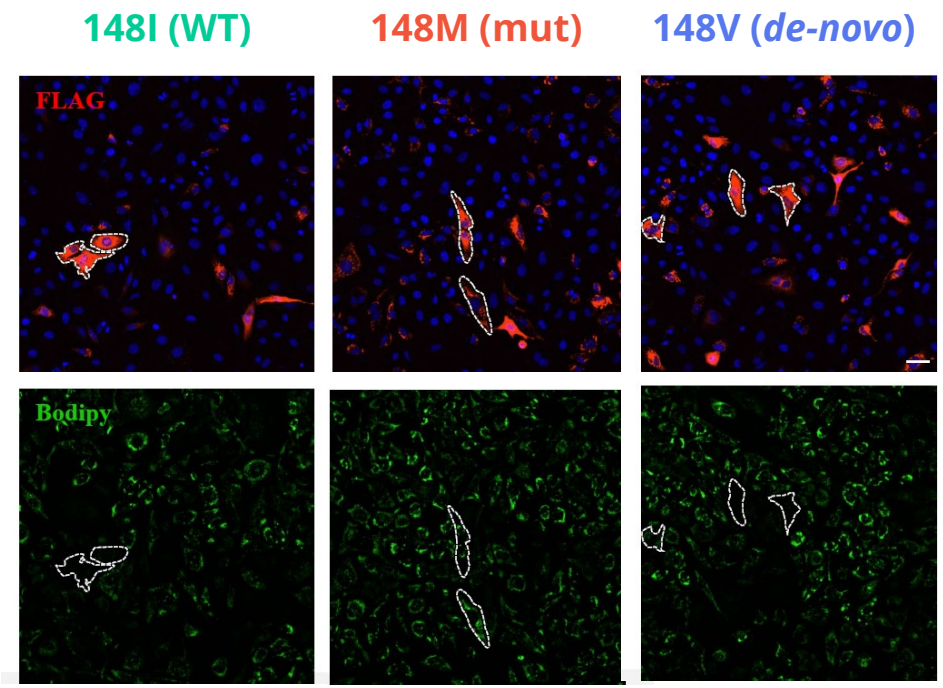
min max

***In silico* analysis of variants**

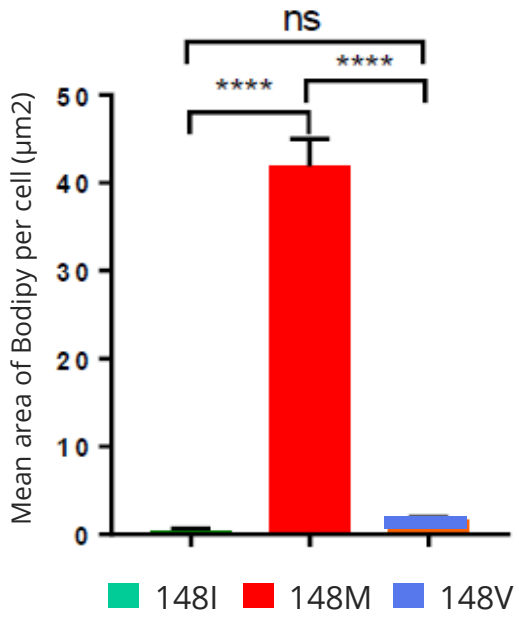
- 148M shows a non-conservative substitution with predicted **functional consequences, with change in binding cavity volume limiting access of substrate to the active site**
- Equivalent potential between Isoleucine (WT) or Valine (Axiomer correction) at location 148 in 3D models
- 148I and 148V predict **no functional consequences** for PNPLA3, with valine expected to behave like isoleucine

# PNPLA3 148V variant has WT-like lipid metabolizing properties

*148I and 148V reports equivalence in lipid droplet sizes*




Hoechst (nuclei), Bodipy (Lipids) and M2 anti-flag (PNPLA3)



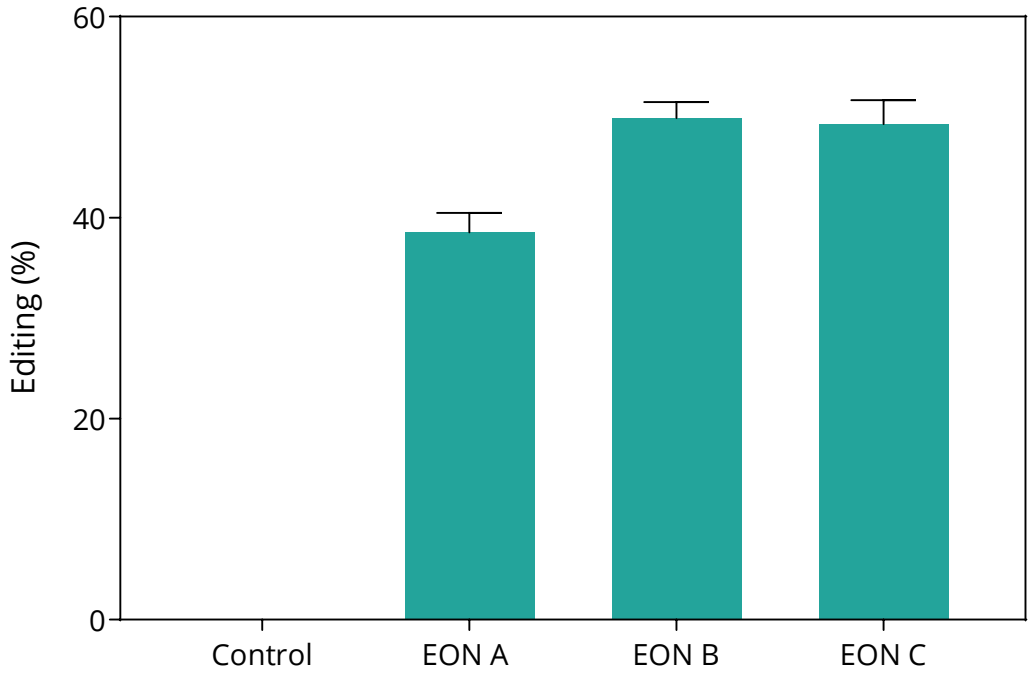

- The wild-type 148I shows smaller lipid droplets, reflecting normal lipid metabolism
- The 148M variant induces significantly larger lipid droplets, consistent with its pathogenic role in lipid metabolism disorders
- The corrected variant 148V results in wild-type like droplet sizes, suggesting a corrective effect on lipid accumulation, similar to 148I

Treatment conditions: HeLa cells, plasmid, transfection, 250uM linoleic acids, 24h, cell lipase activity by IF One-way ANOVA, ****, P<0.0001; Mean, SEM.

# EON mediated PNPLA3 editing leads to over 50% RNA editing and change in lipid droplet

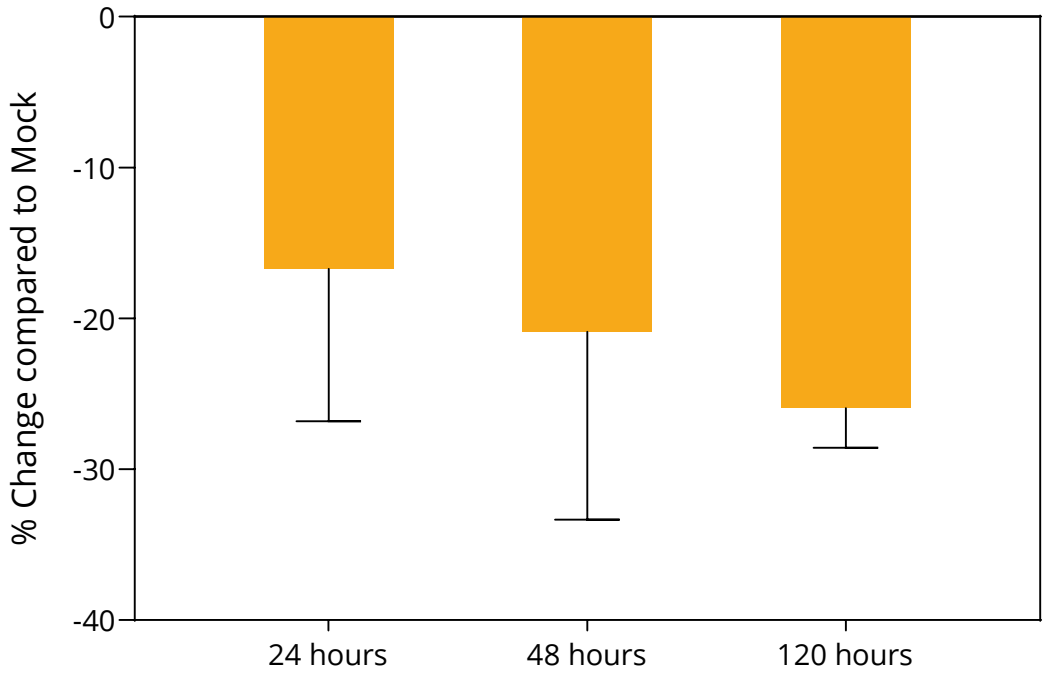
**Editing of PNPLA3 in PHH**

*100nM EON, transfection, 72h, dPCR, mean, SEM, n=3*




**Change in intracellular lipid droplets post PNPLA3 148V EON treatment in HepG2**

*Bodipy/DAPI stainings, 5µM EON, transfection, exposure to linoleic acid, mean, SEM, n=2*



# Summary & next steps

*AX-2911 for MASH*

- **Final optimization of AX-2911 EONs ongoing for clinical candidate selection in 2025**
- **Expected 3-6 monthly dosing interval subcutaneous GalNAc-delivered**
- **Development activities to start in 2025**
- **Start clinical trial in 2026**

# Closing summary

*Daniel A. de Boer*

# ProQR development pipeline

| | TARGET | DISCOVERY | NON-CLINICAL | CLINICAL | NEXT MILESTONE | ESTIMATED POPULATION |
|---|---|---|---|---|---|---|
| **DEVELOPMENT PIPELINE** | | | | | | |
| **AX-0810** *for Cholestatic diseases* | NTCP | | | | CTA filing in Q2 2025 | ~100K patients |
| **AX-2402** *for Rett syndrome* | MECP2 R270X | | | | Candidate selection in 2025 | ~5K patients |
| **AX-1412** *for Cardiovascular disease* | B4GALT1 | | | | Scientific update in mid 2025 | ~200M patients |
| **AX-2911** *for MASH* | PNPLA3 | | | | Candidate selection in 2025 | ~8M patients |
| **DISCOVERY PIPELINE** | | | | | | |
| **AX-1005** *for CVD* | Undisclosed | | | | | ~200M patients |
| **AX-0601** *for obesity and T2D* | Undisclosed | | | | | ~650M patients |
| **AX-9115** *for rare metabolic condition* | Undisclosed | | | | | |
| **AX-2403** *for Rett syndrome* | MECP2 R168X | | | | | ~6K patients |
| **AX-2404** *for Rett syndrome* | MECP2 R255X | | | | | ~5K patients |
| **AX-2405** *for Rett syndrome* | MECP2 R294X | | | | | ~6K patients |
| **AX-2406** *for Rett syndrome* | MECP2 R133H | | | | | |
| **AX-3875** *for rare metabolic & CNS disorder* | Undisclosed | | | | | |
| **AX-4070** *for rare CNS disorder* | Undisclosed | | | | | |
| **PARTNERED PIPELINE** | | | | | | |
| 10 targets (option to expand to 15) | Undisclosed | *Progress undisclosed* | | | | Lilly |

[1]Approximately 100K people affected with Primary Sclerosing Cholangitis and Biliary Atresia in US and EU5. [2]Approximately 200 million people suffer from too high a level of cholesterol in US and EU5. SLC10A1 is the gene that encodes for NTCP protein. CVD: Cardiovascular Diseases, NASH: Nonalcoholic steatohepatitis, T2D: Type 2 Diabetes. | References: Trivedi PJ, et al. Clin Gastroenterol Hepatol. 2022 Aug;20(8):1687-1700.e4; Schreiber RA, et al. J Clin Med. 2022 Feb 14;11(4):999; Tsao CW, et al. Circulation. 2022;145(8):e153–e639. World Health Organization, World Gastroenterology Organization

# Catalyst overview

*4 trial readouts expected in 2025-2026, cash runway into mid-2027*

**AX-0810 for Cholestatic disease**

- CTA submission Q2 2025
- Top-line data Q4 2025

**AX-2402 for Rett Syndrome**

- Clinical candidate selection in 2025
- Anticipated trial start and top-line data in 2026

**AX-1412 for Cardiovascular disease**

- Non-clinical data update in mid 2025

**AX-2911 for MASH**

- Clinical candidate selection in 2025
- Anticipated trial start and top-line data in 2026

**Partnerships**

- Opportunity to earn up to $3.75B in milestones in the Lilly partnership
- Opportunity to receive a $50 M opt-in fee from Lilly for expansion to 15 targets
- Opportunity for other strategic partnerships

# Well positioned
*to advance Axiomer™*

## Clinical trial results across 4 trials in 2025 and 2026 expected

- Clinical PoC data of NTCP trial in 2025
- Up to 4 clinical trials with data readouts in 2025/2026

## Rich discovery pipeline with potential for broad pipeline expansion

- Large number of potential therapeutic applications in discovery pipeline
- Broad applicability beyond current discovery pipeline

## Leading IP position

- Axiomer™ is protected by >20 published patent families
- Continuously investing in expanding IP estate

## Validating Strategic Partnerships

- Eli Lilly collaboration valued up to $3.9B, with opportunity for near-term milestones
- Rett Syndrome Research Trust cofinancing of AX-2402 program
- Selectively form additional partnerships

## Strong balance sheet

- €89.4 million cash and cash equivalents as of end of Q3, plus $82.1 million gross proceeds from October financing
- Cash runway to mid-2027, excluding potential for additional BD-related upside

# Q&A

# Q&A

**Daniel de Boer**
*Founder and*
*Chief Executive Officer*

**René Beukema**
*Chief Corporate*
*Development Officer*

**Gerard Platenburg**
*Chief Scientific Officer*

ProQR®

# IT'S IN OUR RNA