26th JSSX Annual Meeting in Hiroshima
Young Investigator's Award
Nov 17th, 2011

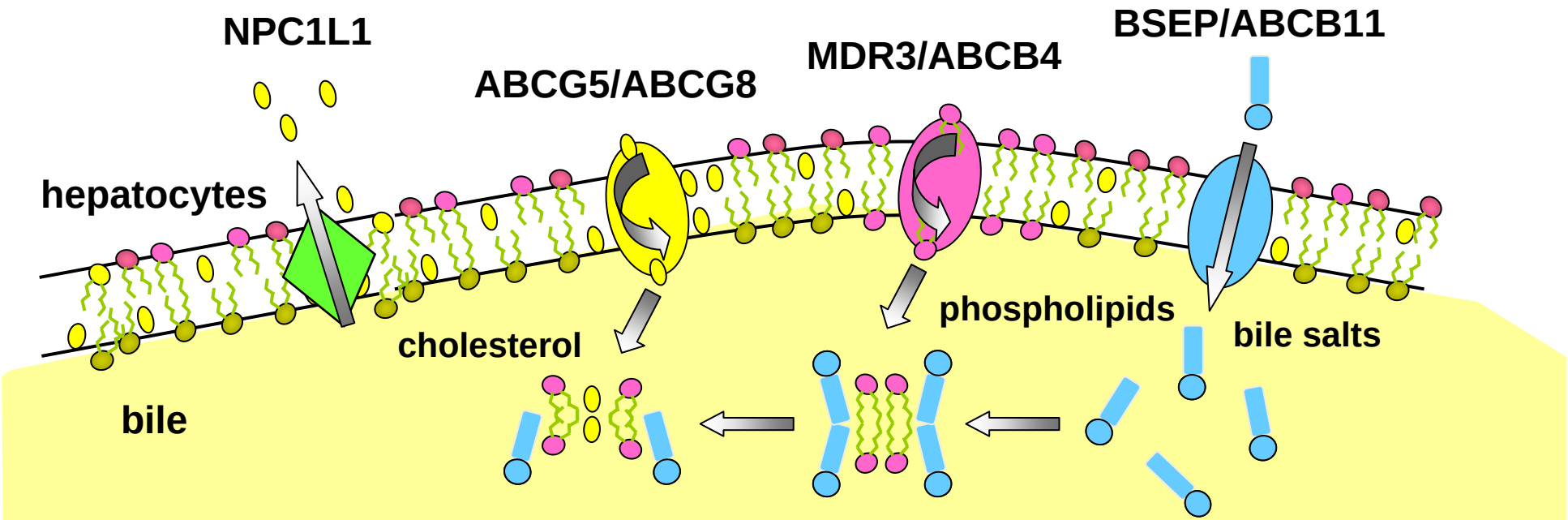
Regulatory mechanisms of bile lipids transport in the small intestine and liver

消化管および肝臓におけるトランスポーターを介した胆汁脂質動態制御機構の解析

Tappei Takada

Department of Pharmacy, the University of Tokyo Hospital

Bile lipids transporters on the canalicular membrane



JPET (2007)
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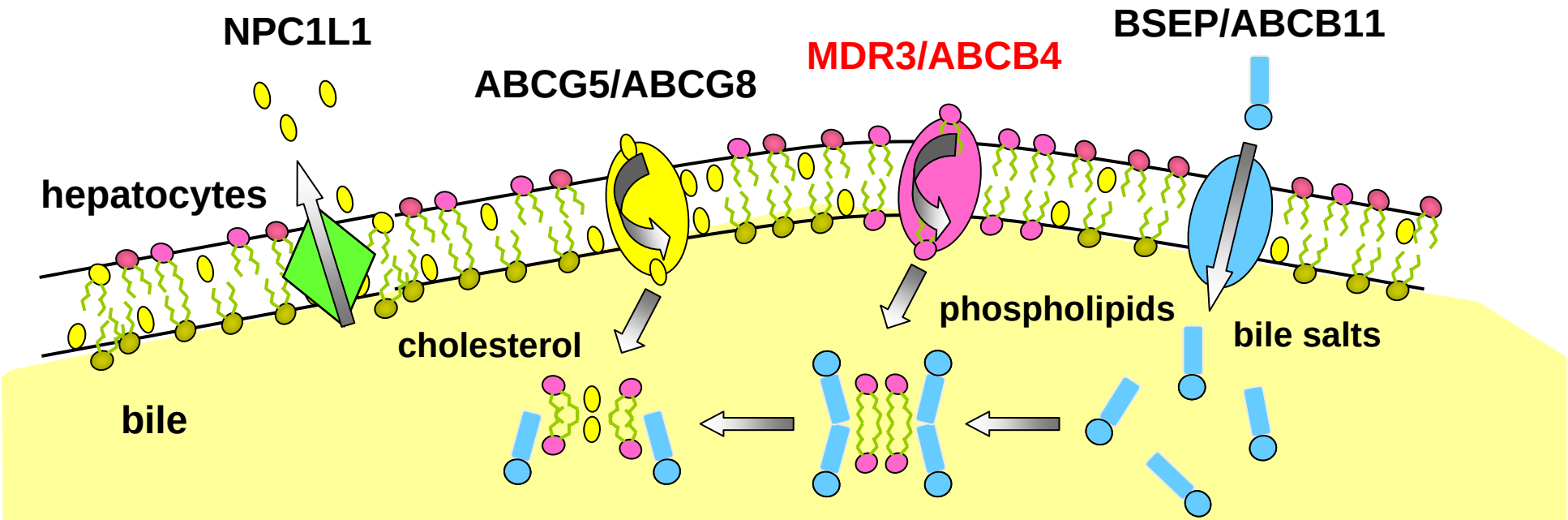

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***Mol Pharmacol* (2011)**

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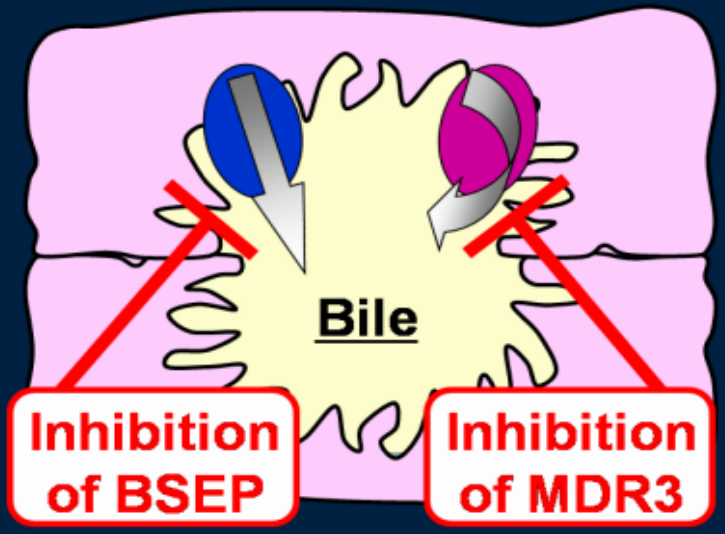
Hypothesis

Drug-induced cholestasis via the inhibition of BSEP and MDR3



BSEP and MDR3 play essential roles in biliary secretion, and their genetic disruptions cause progressive familial intrahepatic cholestasis (PFIC)

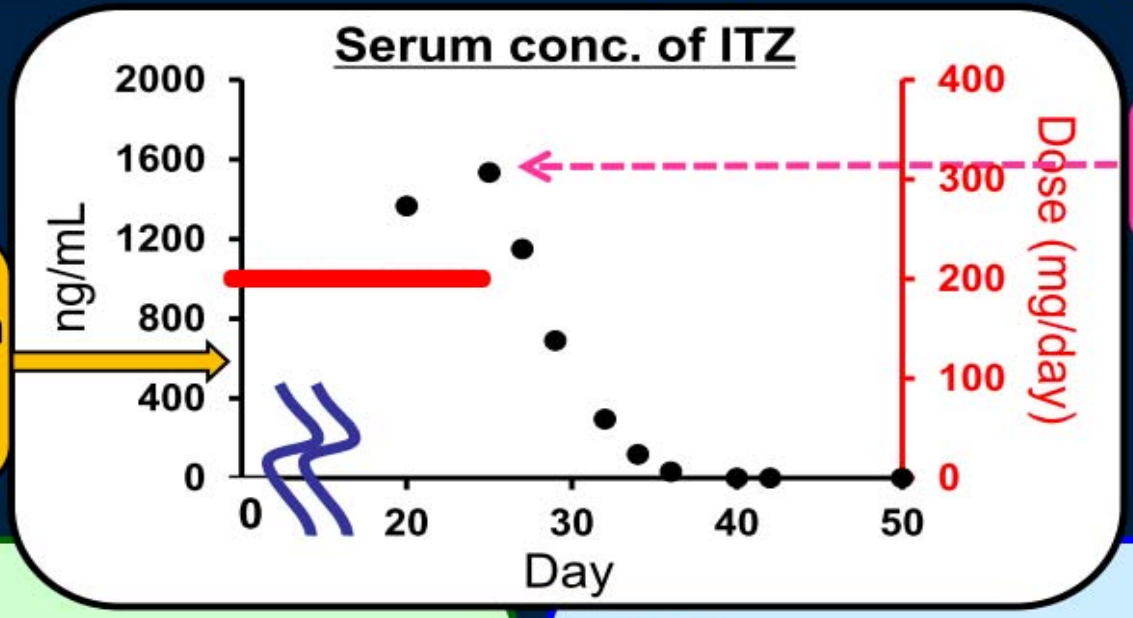
- Some cholestatic drugs have been reported as inhibitors of BSEP, although their inhibitory effects have been usually observed at much higher drug concentration than clinical situations.
- There has been no report studying inhibitiory effects on MDR3 by cholestatic drugs.

We should examine MDR3-related mechanism, in addition to BSEP.

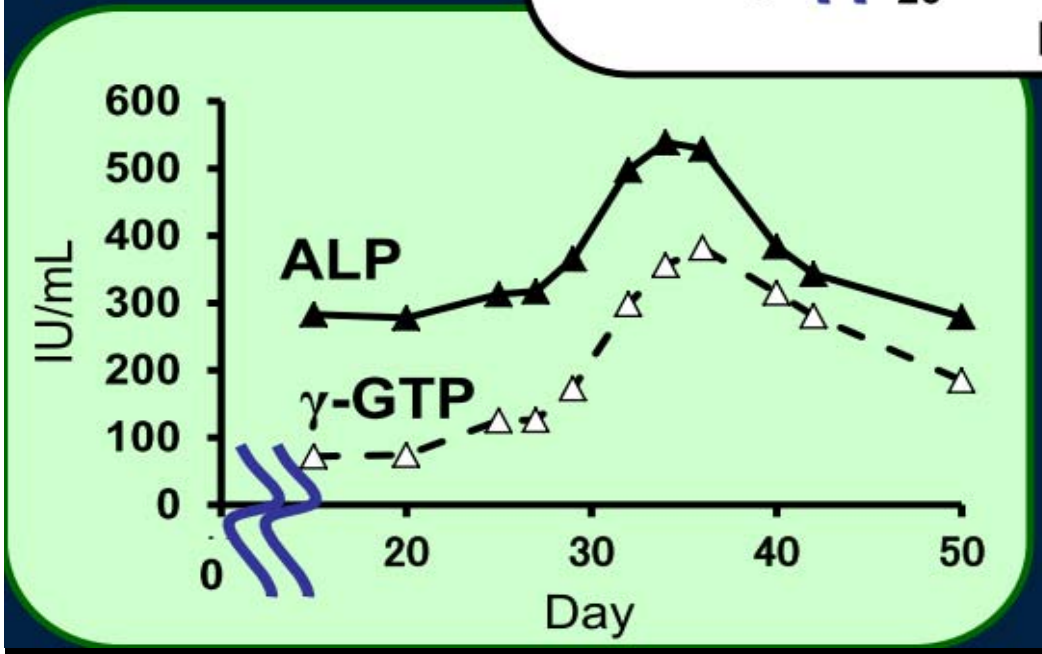
Case of itraconazole-induced liver injury in Univ. Tokyo Hospital

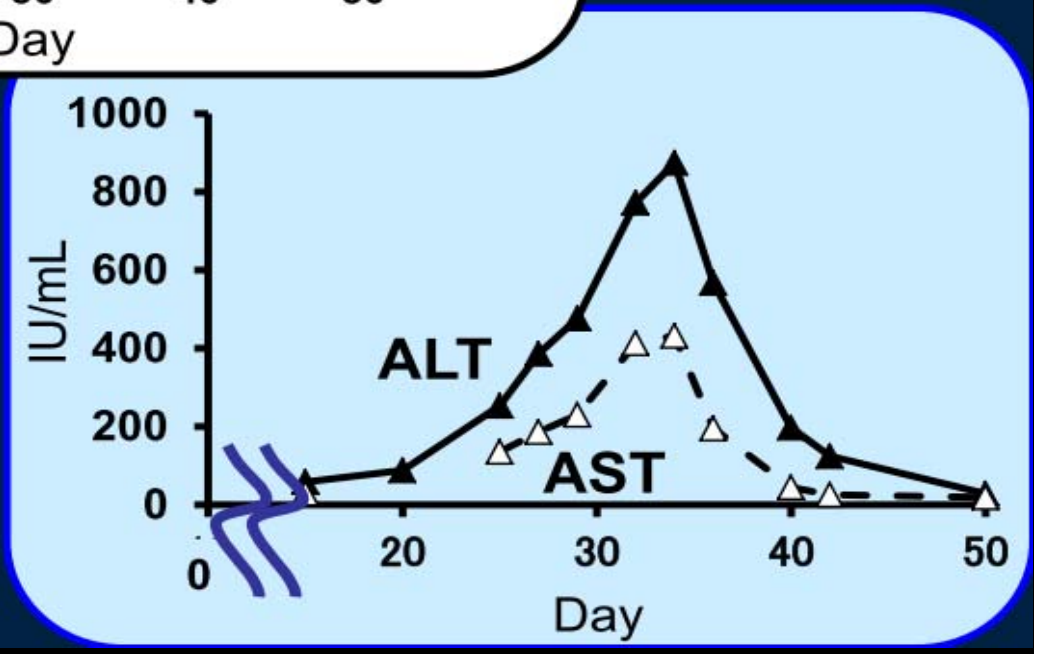
On Day 1, ITZ oral solution was started (200mg/day)

Average conc. in Japanese population 500 ng/mL (trough conc.)



1534 ng/mL (trough conc.)





Purpose of the study

To investigate the effect of ITZ on biliary secretion

Analysis focusing on functions of BSEP and MDR3

<In vivo>

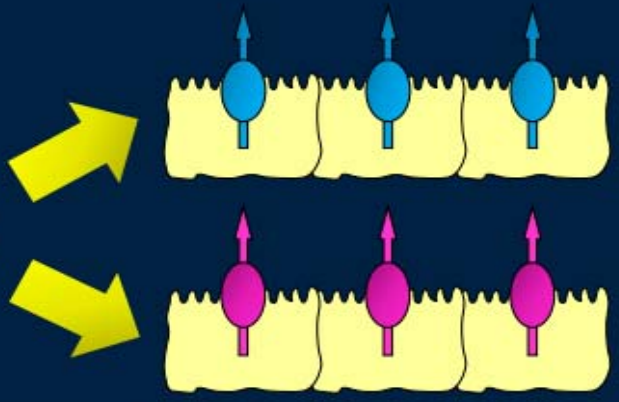
Administration of ITZ

SD rat

Effect on the biliary secretions of bile acids and phospholipids

<In vitro>

Incubation with ITZ

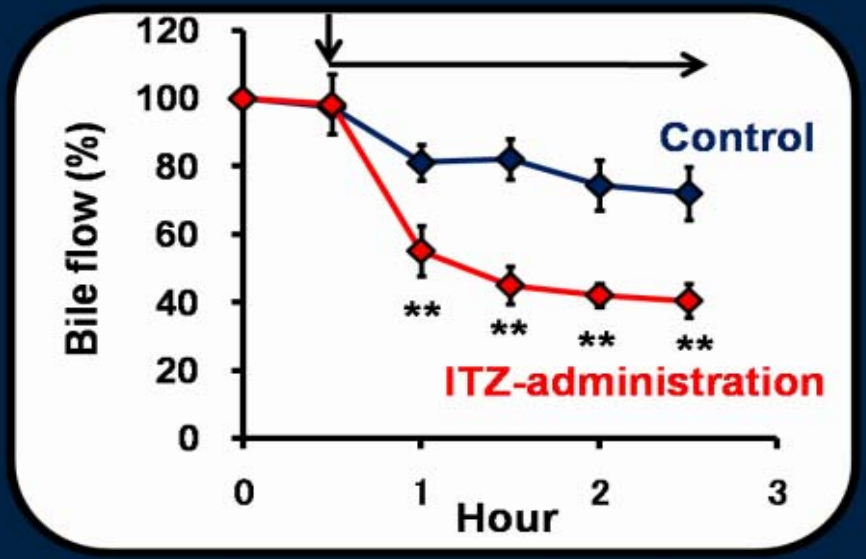


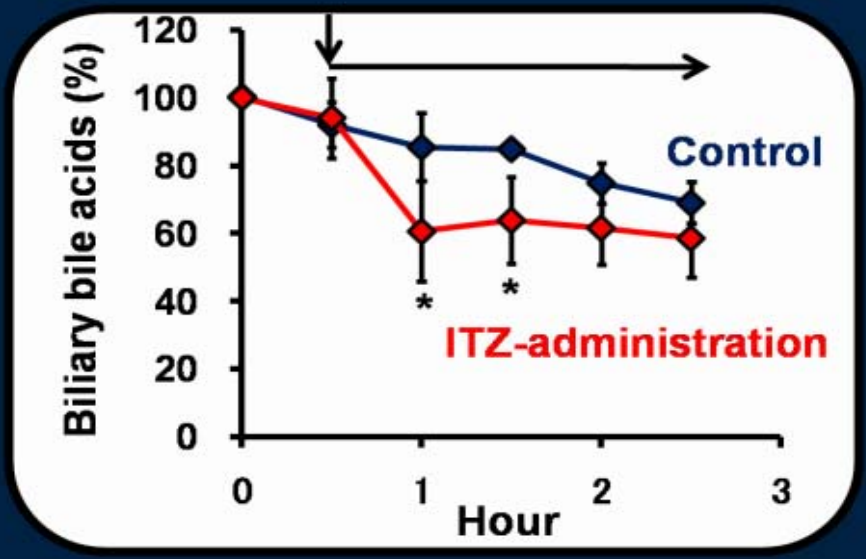
Effect on BSEP

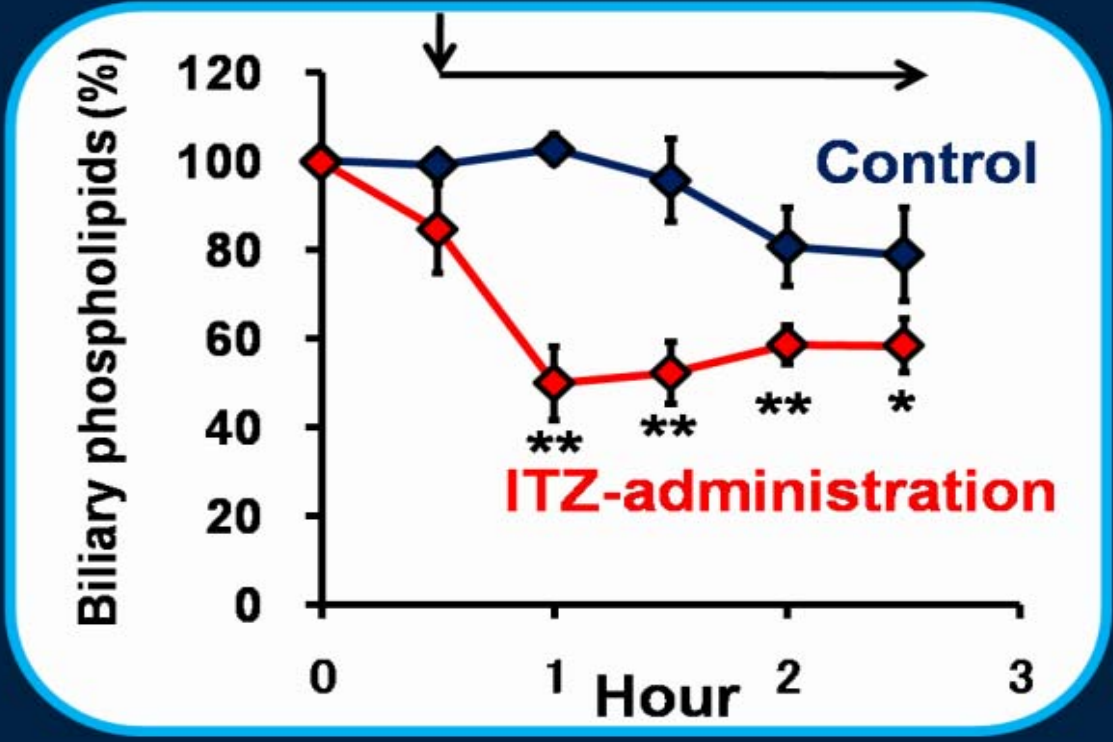
Effect on MDR3

Transporters expressed in LLC-PK1 cells

Effect of ITZ on biliary secretion *in vivo*







Biliary phospholipids were drastically decreased in ITZ-administered rats

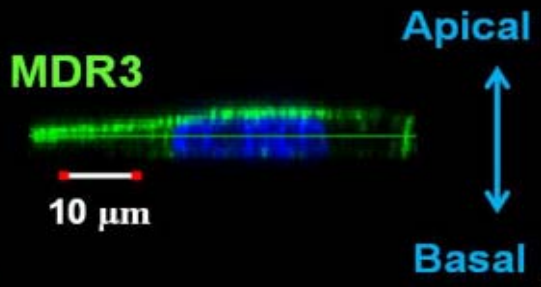
→ Possibility of the inhibition of MDR3

Mean±SD (n=4). *p<0.05, **p<0.01

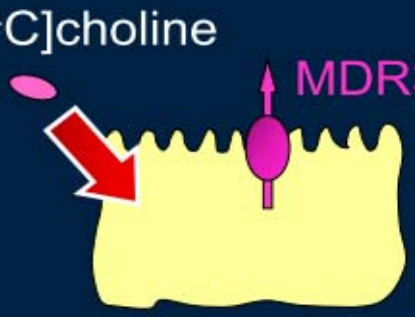
MDR3-mediated efflux of phosphatidylcholine *in vitro*

LLC-PK1 cells were infected with recombinant adenoviruses and MDR3-mediated efflux of phosphatidylcholine (PC) was studied

Apical localization of MDR3 in LLC-PK1 cell



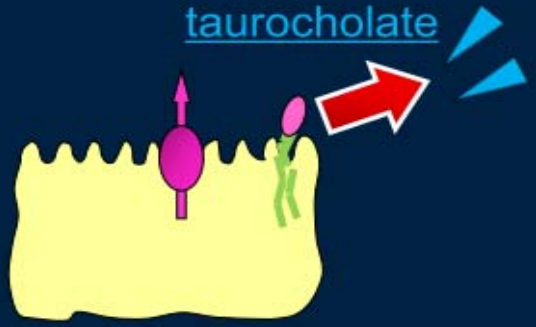
① Addition of [^{14}C]choline



② Conversion to [^{14}C]PC

③ MDR3-mediated translocation of PC

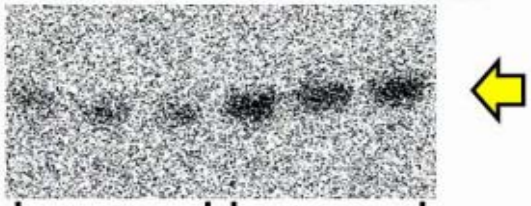
④ Extraction of PC by taurocholate



Effect of ITZ on MDR3-mediated efflux of PC

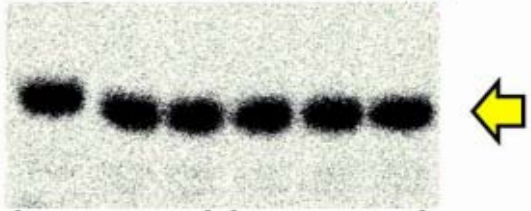
Separation of PC by thin-layer chromatography

PC exported to medium (A)



mock (n=3) MDR3 (n=3)

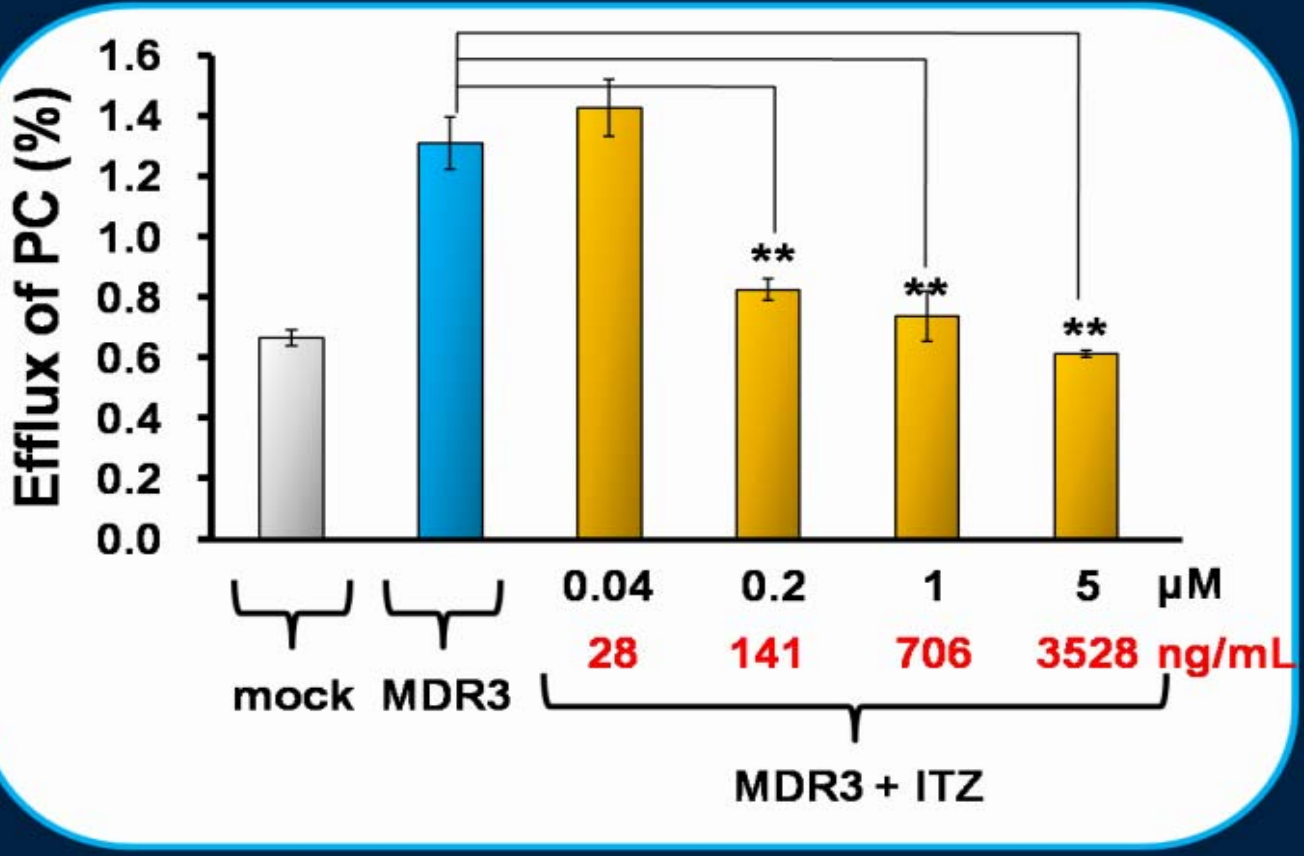
PC remained in cells (B)



mock (n=3) MDR3 (n=3)

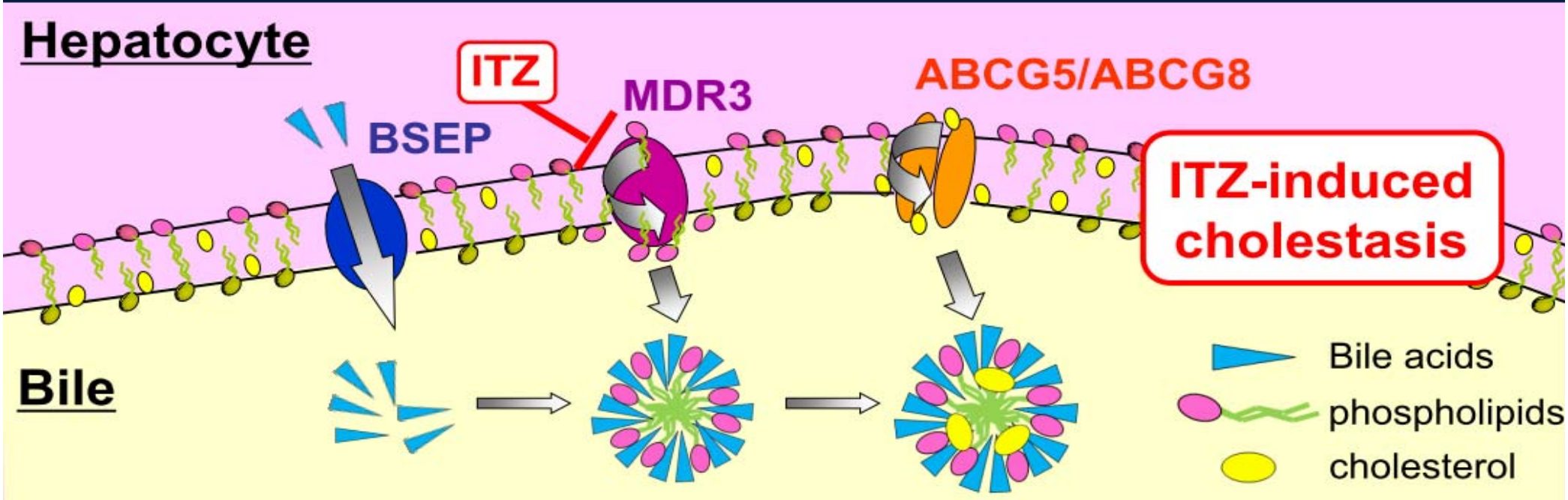
< Efflux of PC (%) >

$$\frac{(A)}{(A)+(B)} \times 100\,(\%)$$



Inhibitory effect on MDR3 was observed in cells treated with ITZ

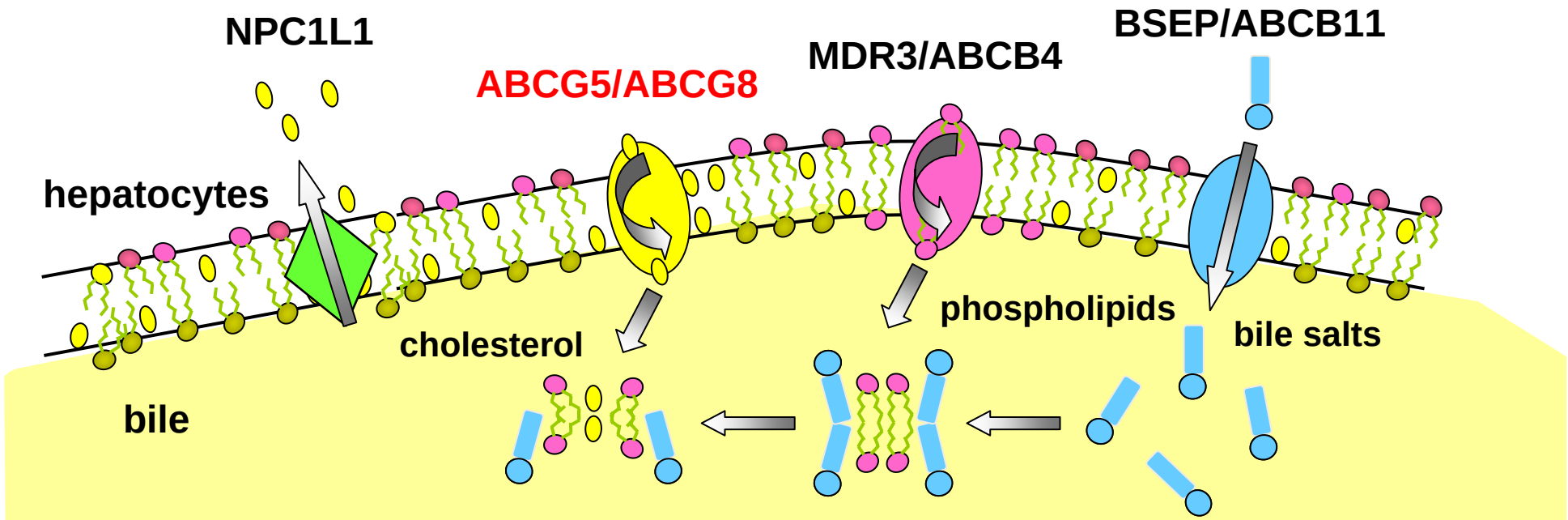
- We found two cases of itraconazole (ITZ)-induced liver injury.
- Biliary phospholipids were decreased in ITZ-administered rats.
- ITZ showed an inhibitory effect on MDR3, but not on BSEP *in vitro*.



Inhibitory effect of ITZ on MDR3-mediated biliary secretion of phospholipids may be involved in ITZ-induced cholestasis.

Yoshikado T, Takada T, et al., *Mol Pharmacol.* (2011)

Bile lipids transporters on the canalicular membrane



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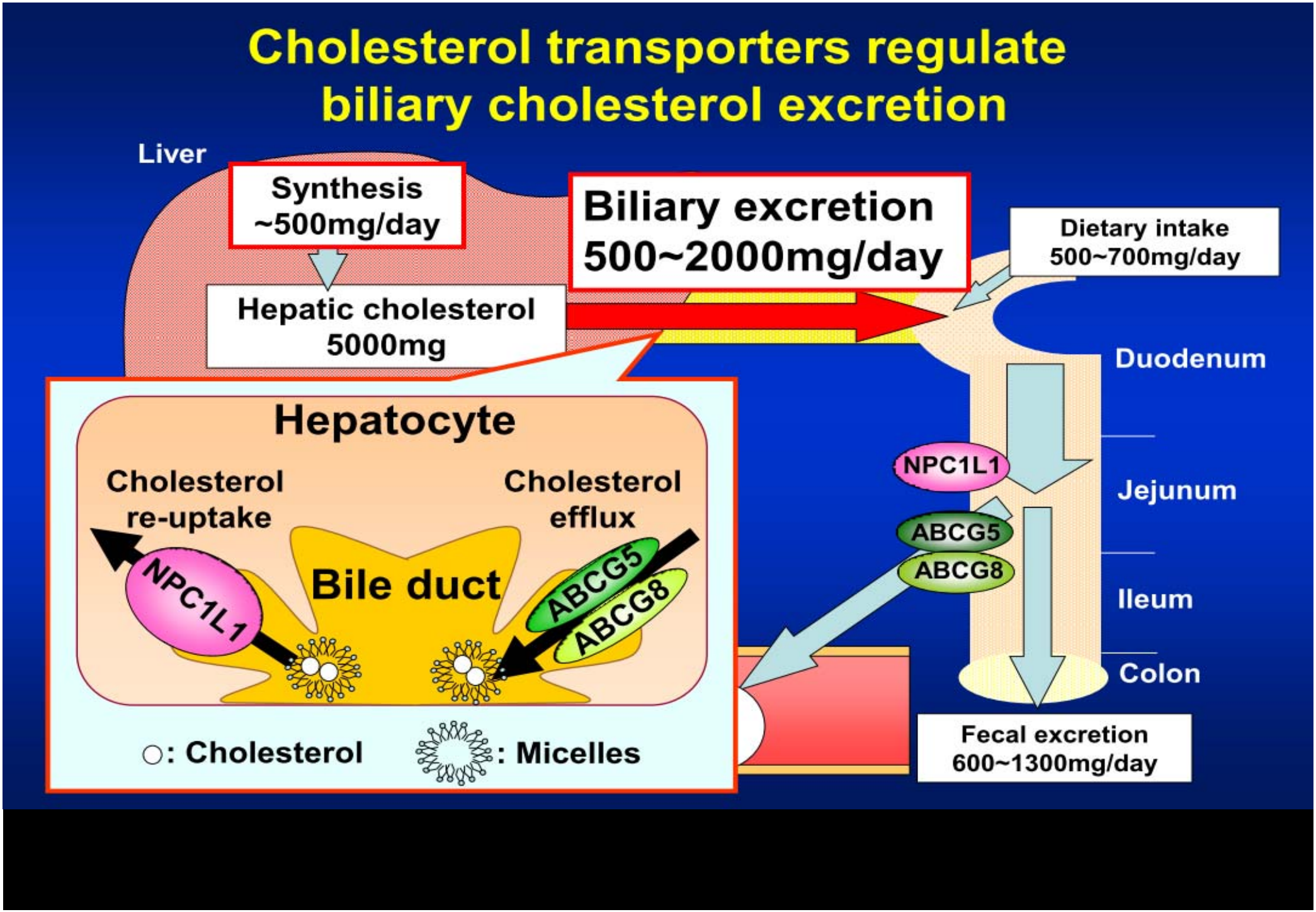
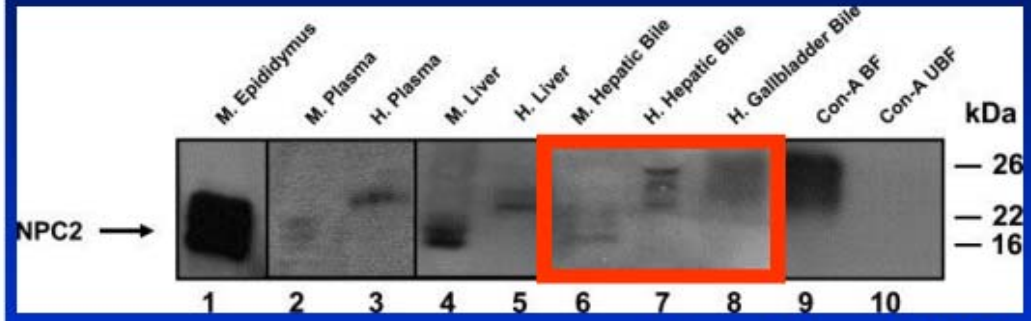

Cholesterol transporters regulate biliary cholesterol excretion
Liver
Synthesis ~500mg/day
Hepatic cholesterol 5000mg
Biliary excretion 500~2000mg/day
Dietary intake 500~700mg/day
Duodenum
Jejunum
Ileum
Colon
NPC1L1
ABCG5
ABCG8
Hepatocyte
Cholesterol re-uptake
Cholesterol efflux
Bile duct
NPC1L1
ABCG5
ABCG8
○: Cholesterol
: Micelles
Fecal excretion 600~1300mg/day

NPC2 is secreted into bile

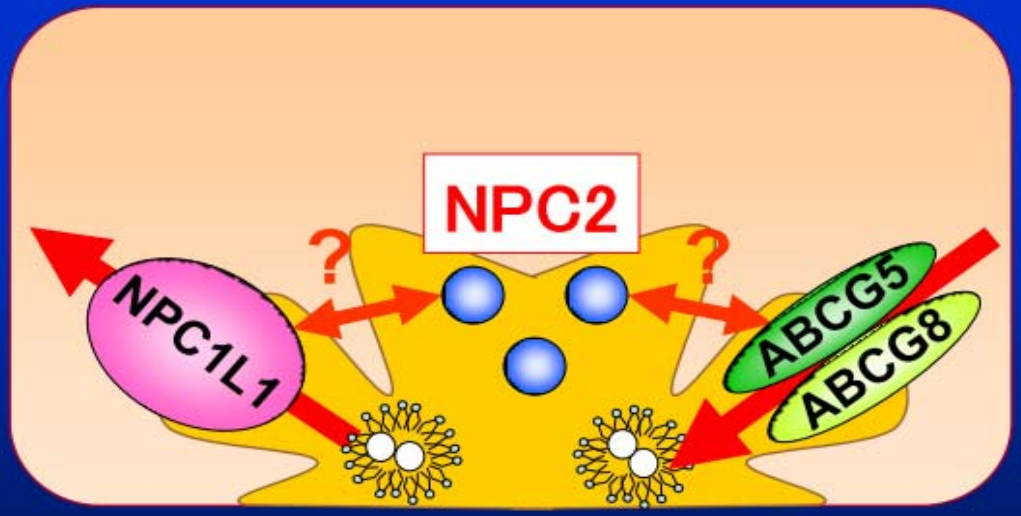


(*Hepatology*. 2006)

Hepatic NPC2 is secreted into bile.

↓ But

Function of biliary NPC2 has not been clarified yet.



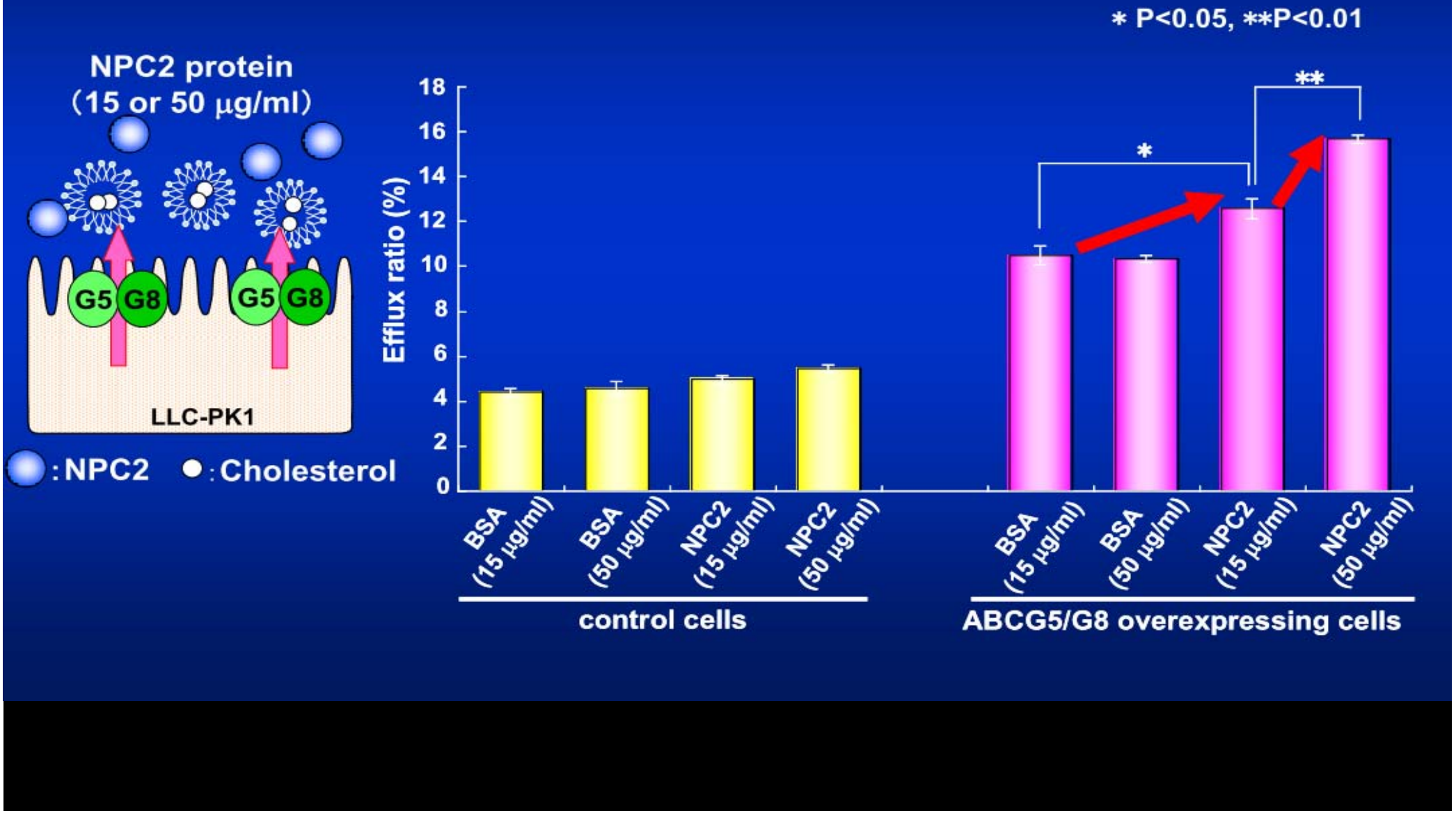
:NPC2 ●: Cholesterol : Micelles

Focusing points

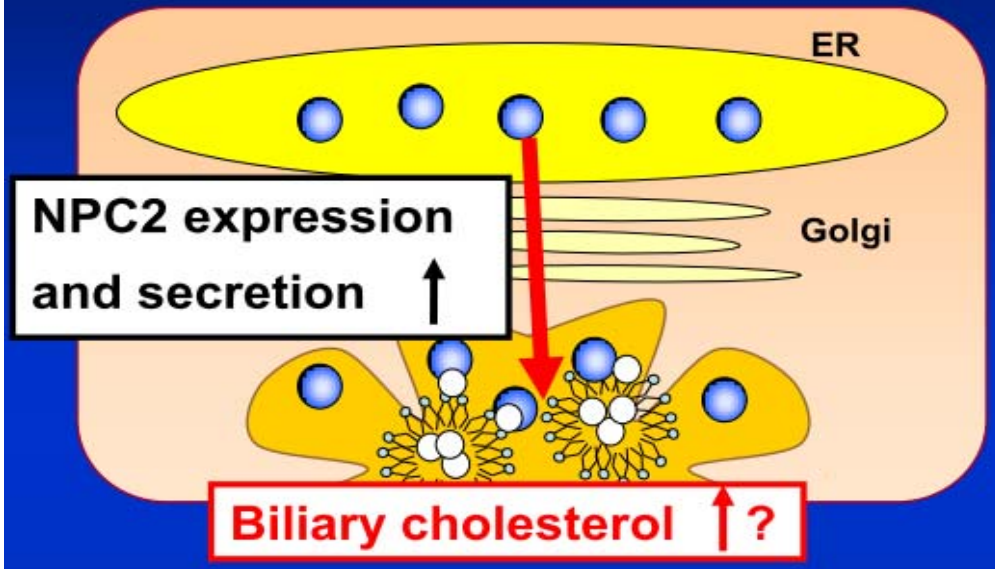
(1) Effect of secreted NPC2 on NPC1L1-mediated cholesterol uptake.

(2) Effect of secreted NPC2 on ABCG5/G8-mediated cholesterol secretion.

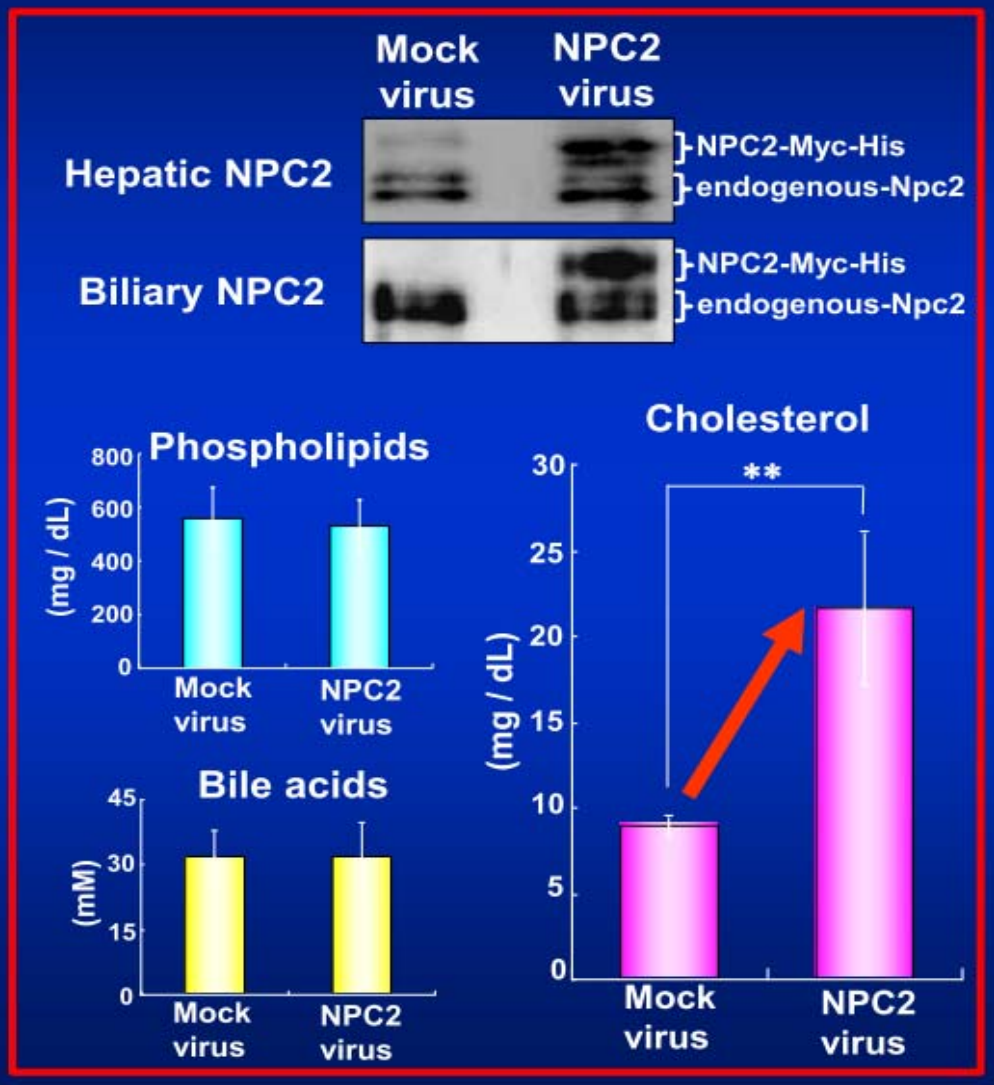
Secreted NPC2 stimulates ABCG5/G8-mediated cholesterol efflux



NPC2 stimulates biliary cholesterol secretion



Hepatic NPC2 overexpressing mouse



Can NPC2 stimulate ABCG5/G8-mediated biliary cholesterol secretion?

Wild-type mouse

NPC2 expression and secretion ↑

ABCG5

ABCG8

Biliary cholesterol ↑

Activity of ABCG5/G8 ↑ ?

?

ABCG5/G8 KO mouse

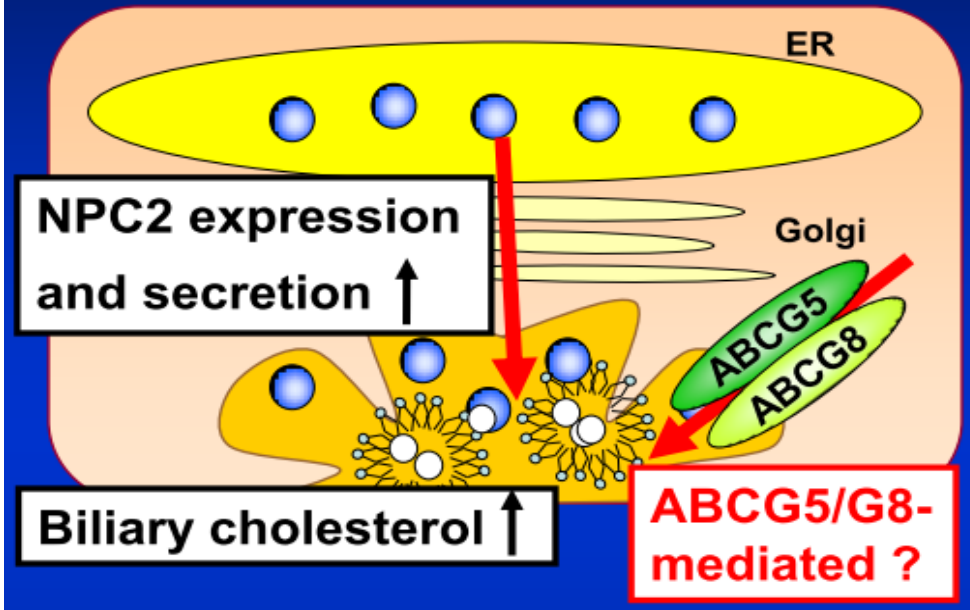
NPC2 expression and secretion ↑

Biliary cholesterol ↑ ?

Deficiency of ABCG5/G8

Analyzing the effect of NPC2 on biliary cholesterol secretion in ABCG5/G8 knock out mouse

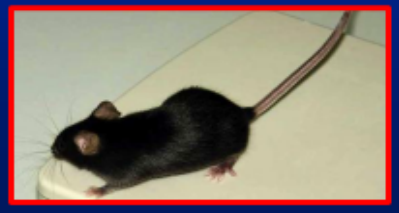
ABCG5/G8 is necessary for the stimulatory effect of NPC2 on biliary cholesterol secretion

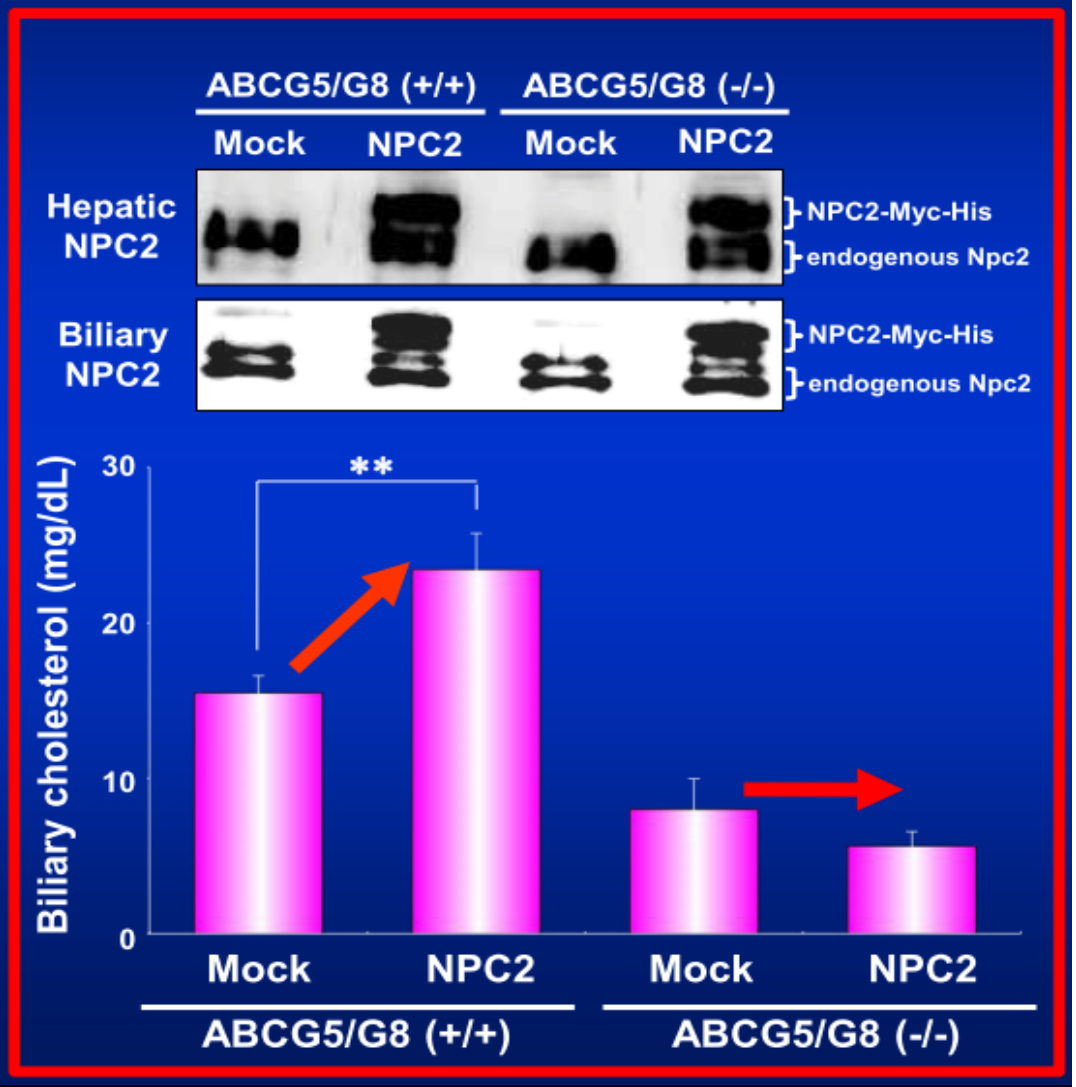


Hepatic NPC2 over-expressing

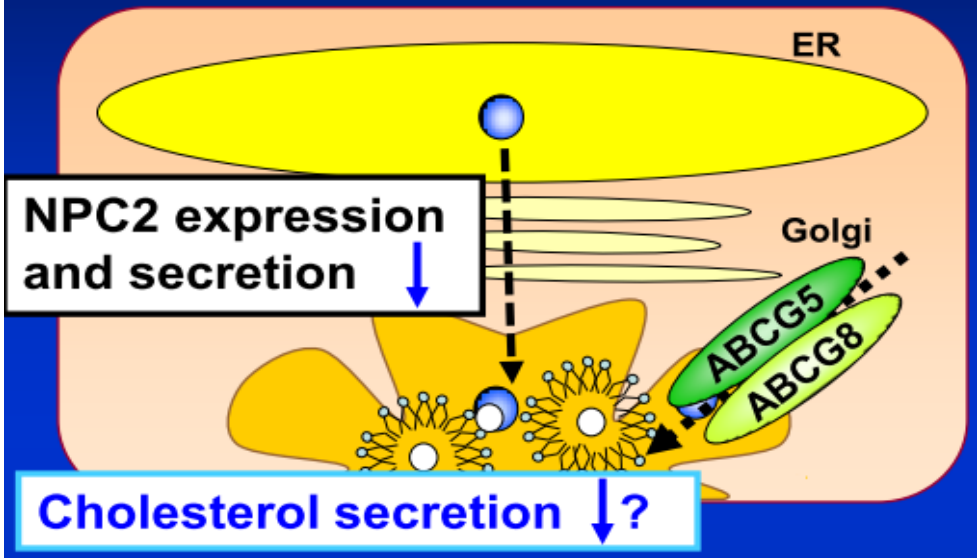
wild-type mouse
or
ABCG5/G8 KO mouse

NPC2 expressing adenovirus →



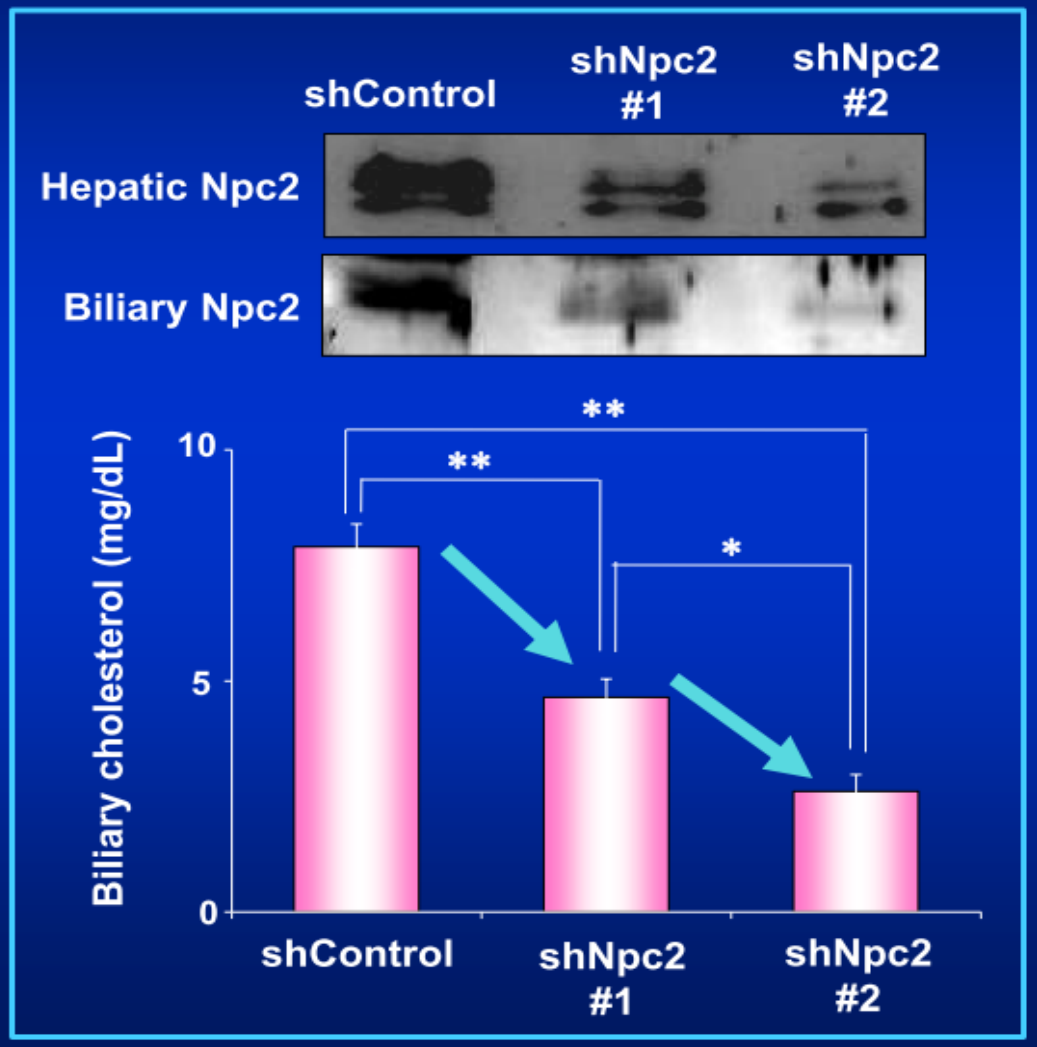


Physiological function of biliary NPC2 as a stimulator of biliary cholesterol secretion

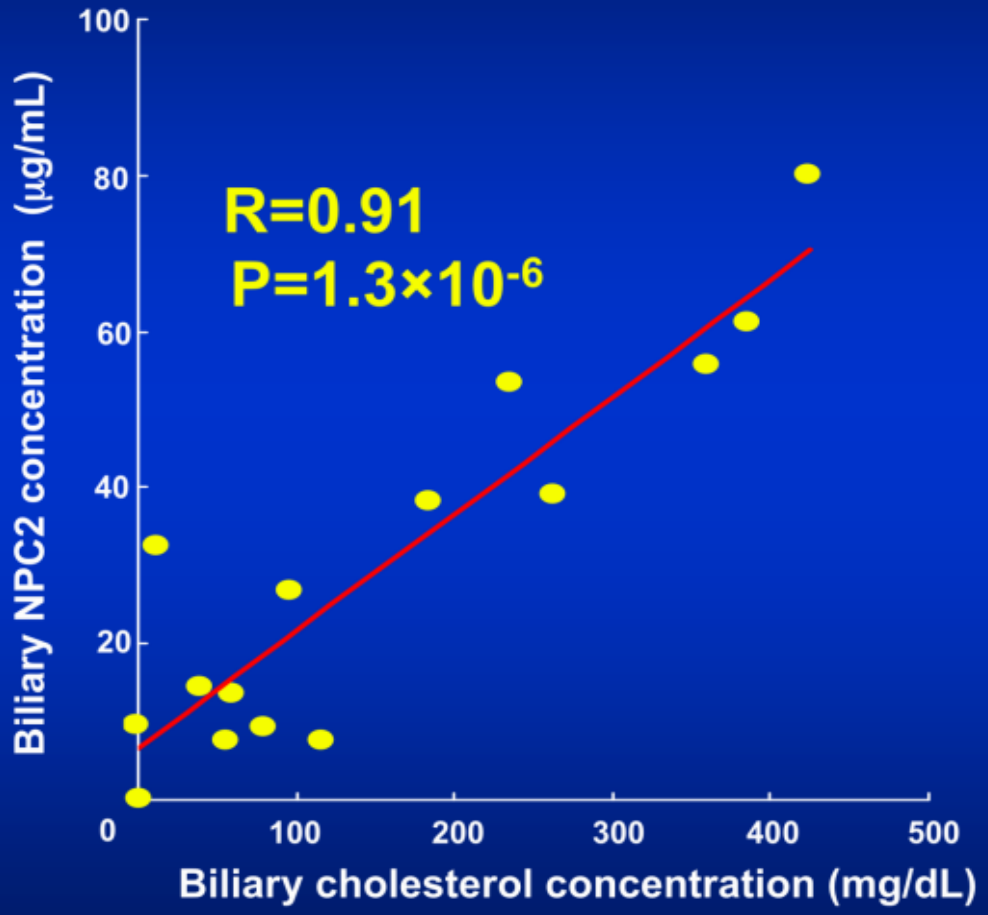


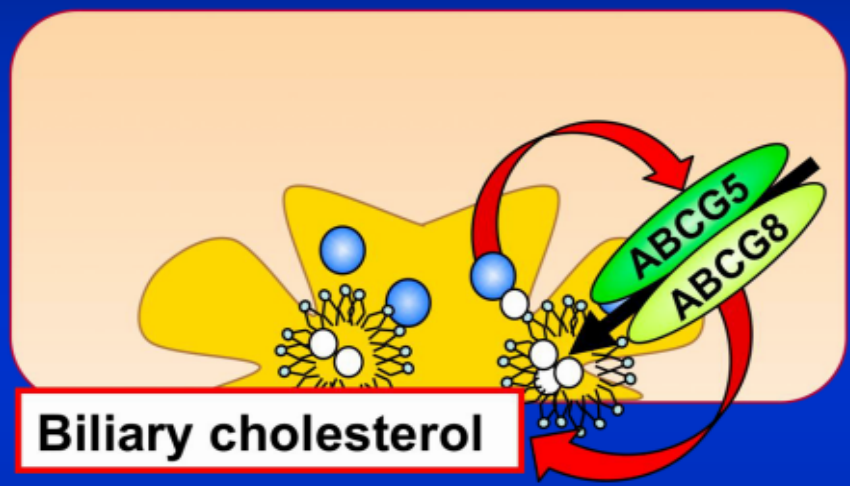
Hepatic Npc2 knockdown mouse

shNpc2 expressing adenovirus



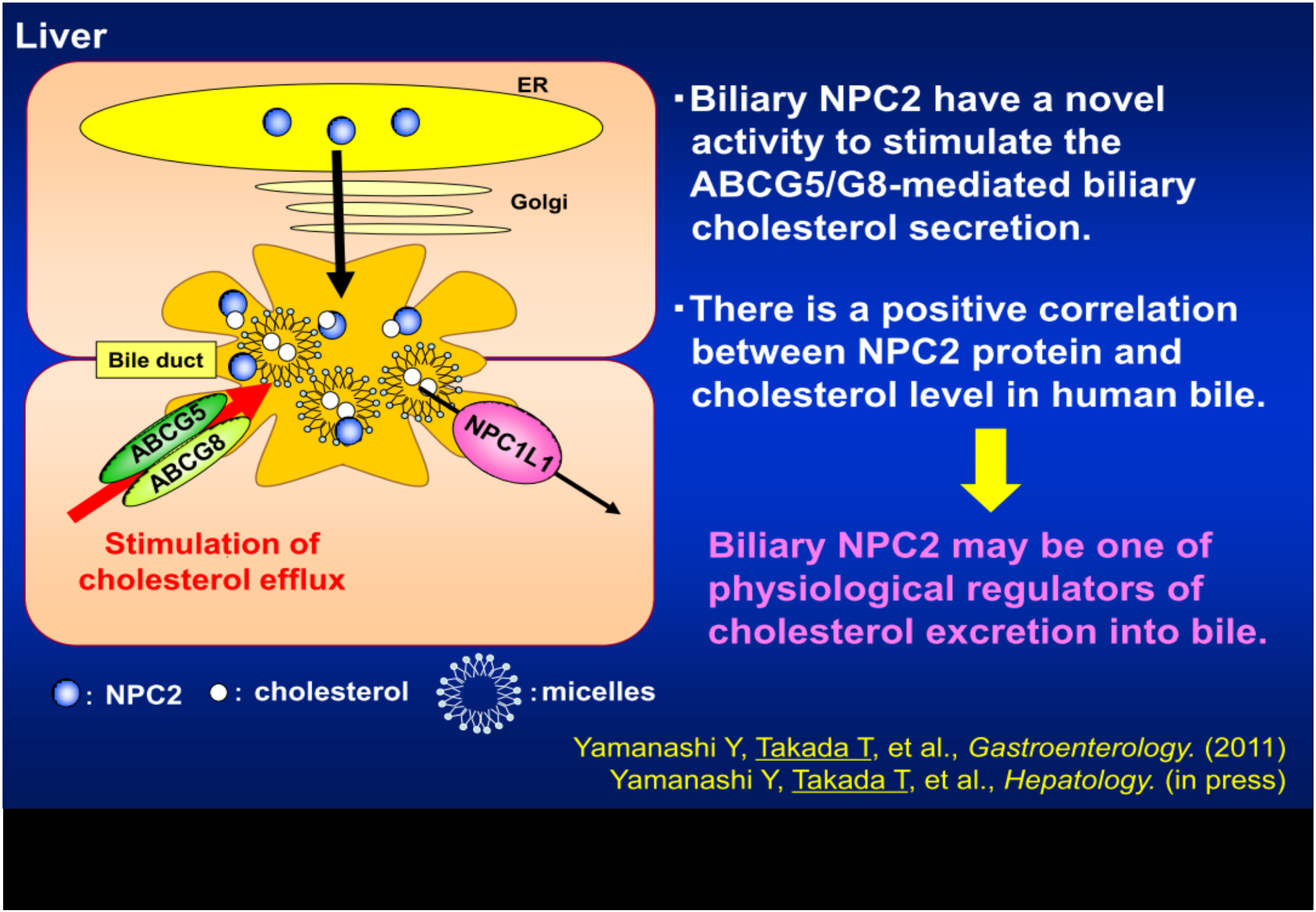
Correlation between NPC2 amount and cholesterol concentration in human bile





There is a positive correlation between NPC2 amount and cholesterol concentration in human bile.

Human bile specimens were kindly provided by Dr. J Shoda, Tsukuba University

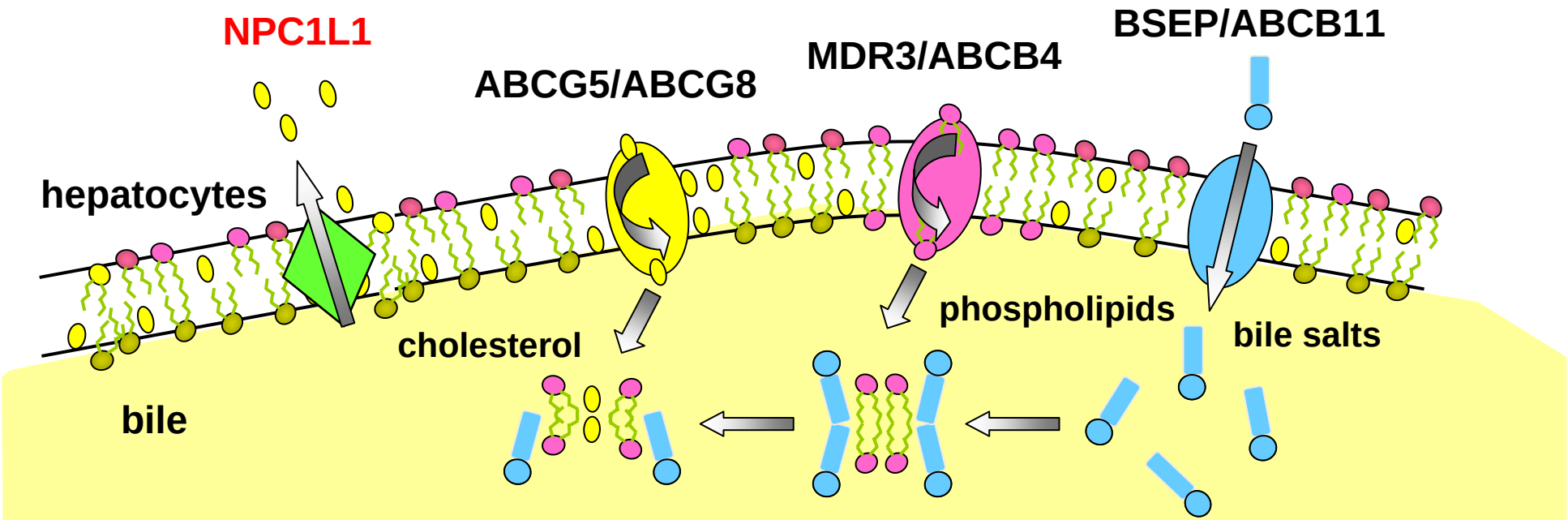


- Biliary NPC2 have a novel activity to stimulate the ABCG5/G8-mediated biliary cholesterol secretion.
- There is a positive correlation between NPC2 protein and cholesterol level in human bile.

Biliary NPC2 may be one of physiological regulators of cholesterol excretion into bile.

Yamanashi Y, Takada T, et al., *Gastroenterology.* (2011)
Yamanashi Y, Takada T, et al., *Hepatology.* (in press)

Bile lipids transporters on the canalicular membrane



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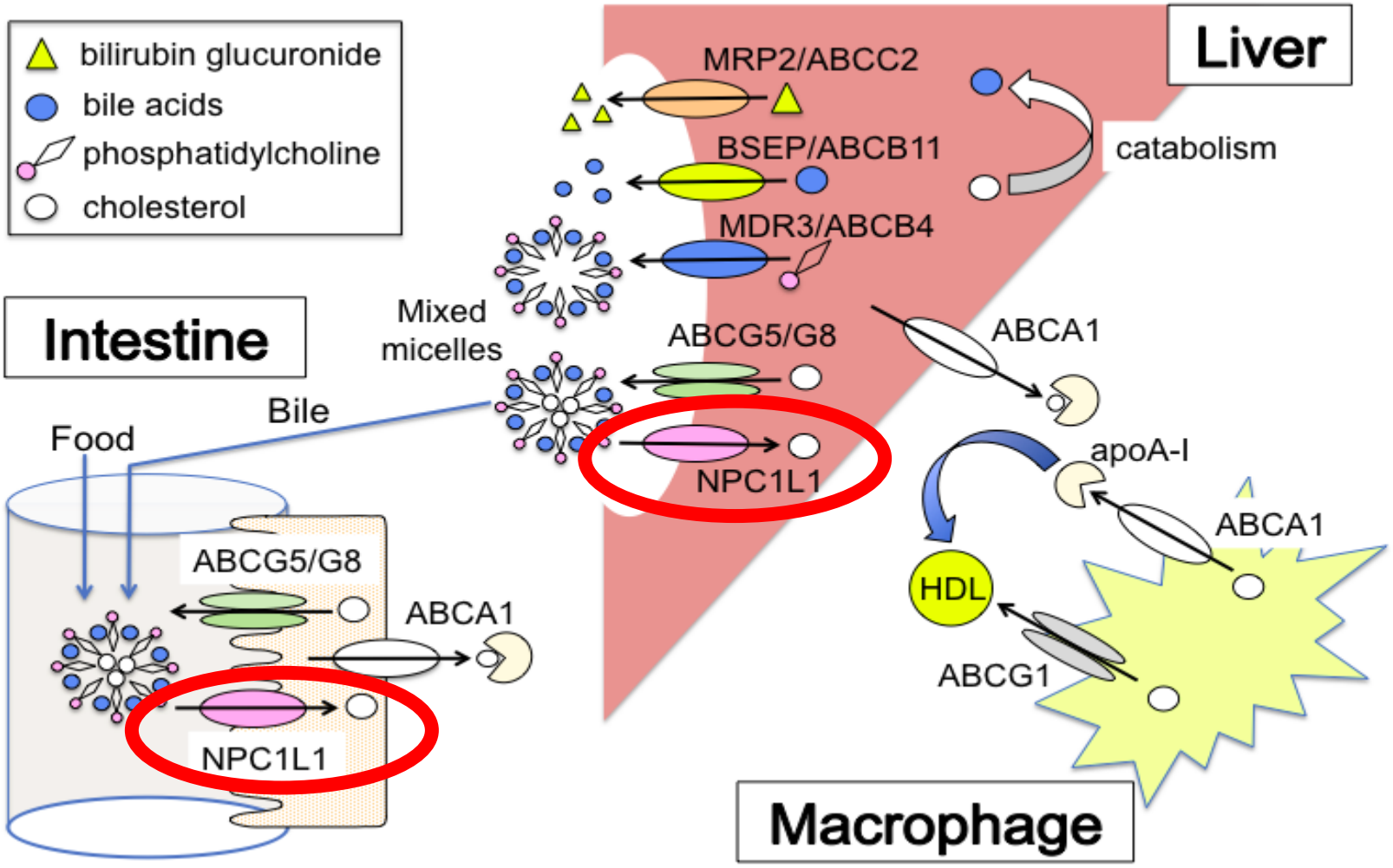
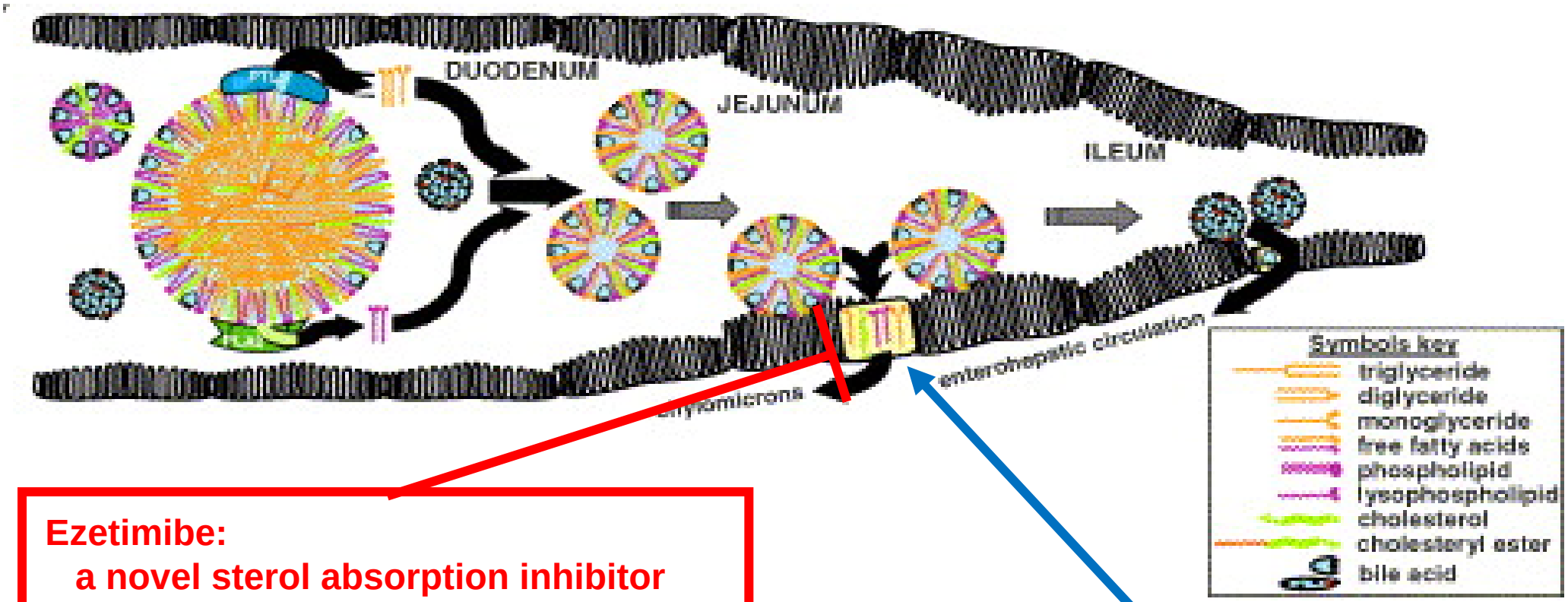
Physiological function of lipid transporters

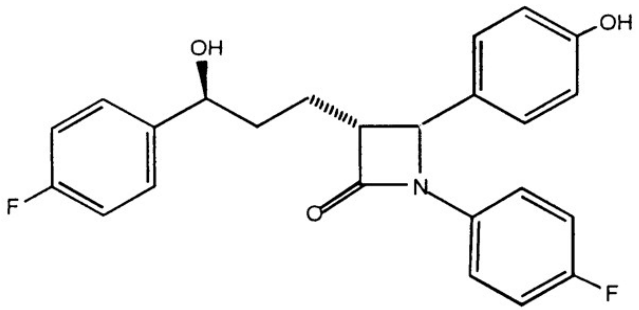


Yamanashi Y, Takada T, Suzuki H
IDENSHI-IGAKU-MOOK (2011)

The process of intestinal absorption of dietary lipids.



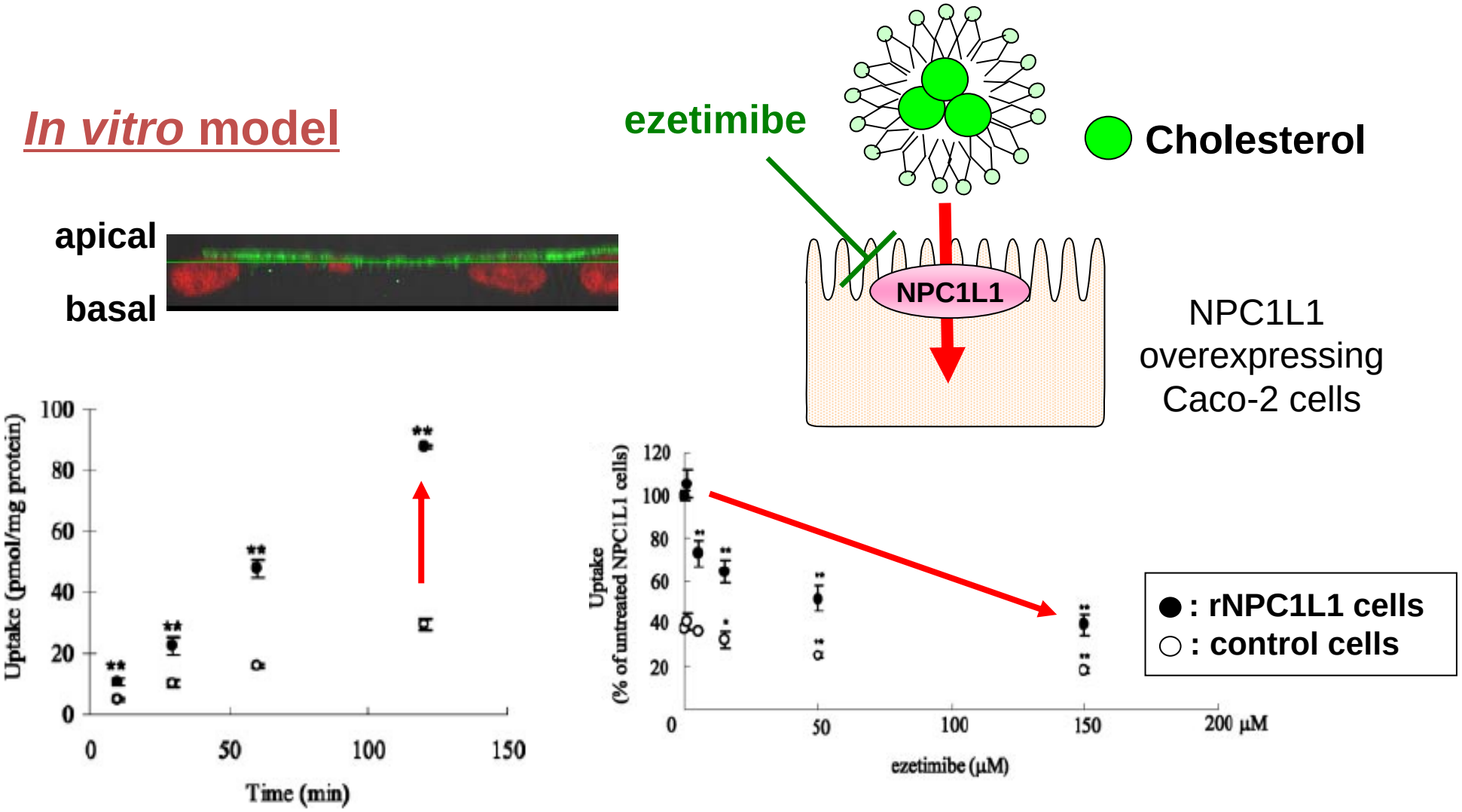
Ezetimibe:
a novel sterol absorption inhibitor



Niemann-Pick C1-like 1 (NPC1L1):
a cholesterol transporter

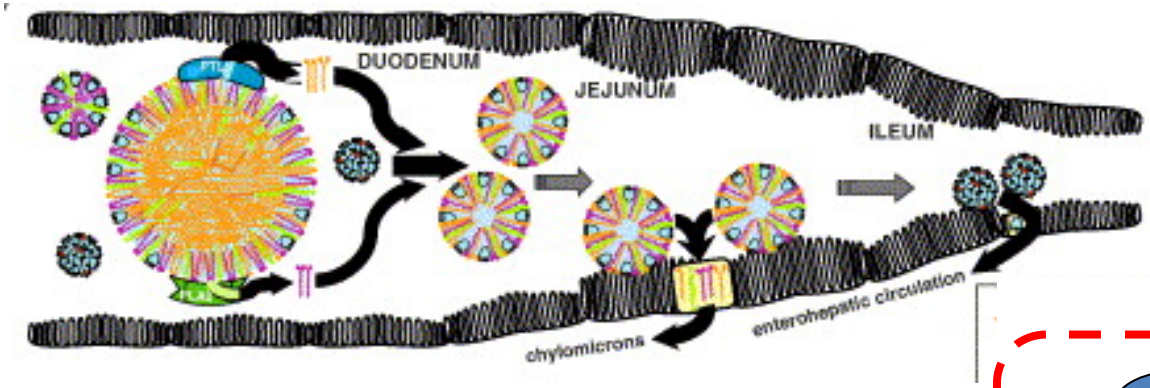
Semin Cell Dev Biol (2005)

In vitro model



Cholesterol uptake is increased by rat NPC1L1 overexpression and the uptake is ezetimibe-sensitive.

Yamanashi Y, Takada T, et al., *JPET* (2007)



Cholesterol

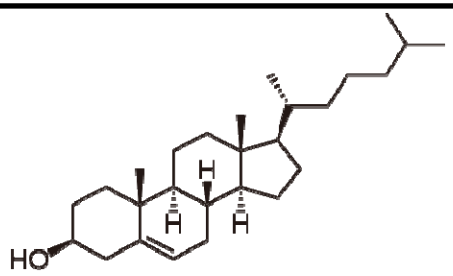
Phospholipids

Bile acids

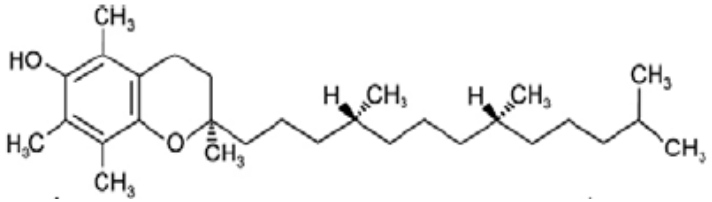
Fat-soluble vitamins

Fat-soluble drugs

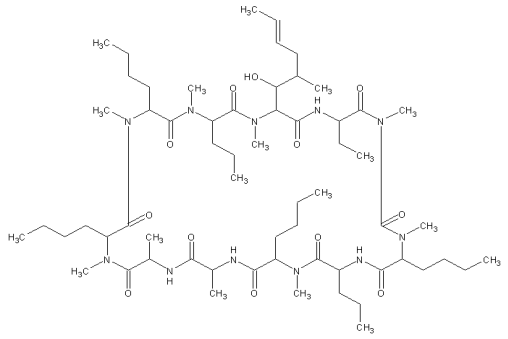
Cholesterol



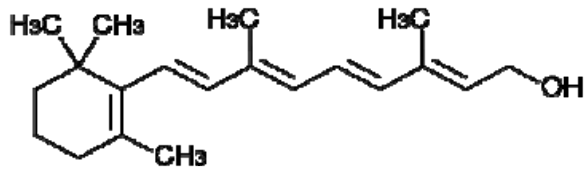
α-Tocopherol (vitamin E)



Cyclosporin A (fat-soluble drug)

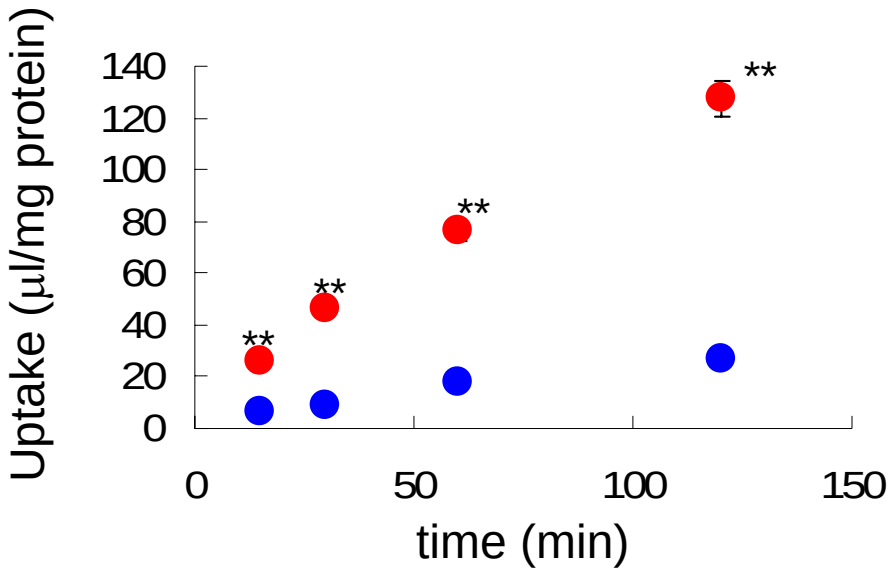


Retinol (vitamin A)

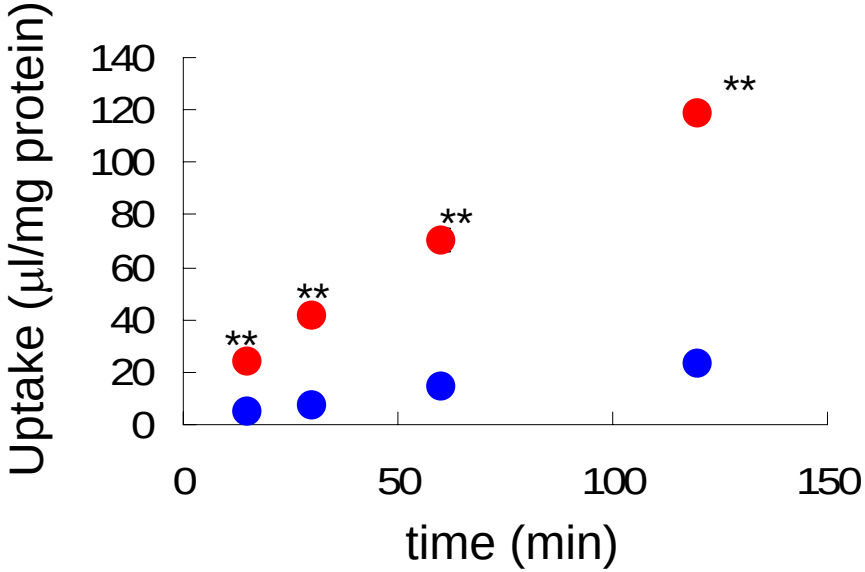


Time profiles of NPC1L1-mediated uptake

Cholesterol



α-Tocopherol



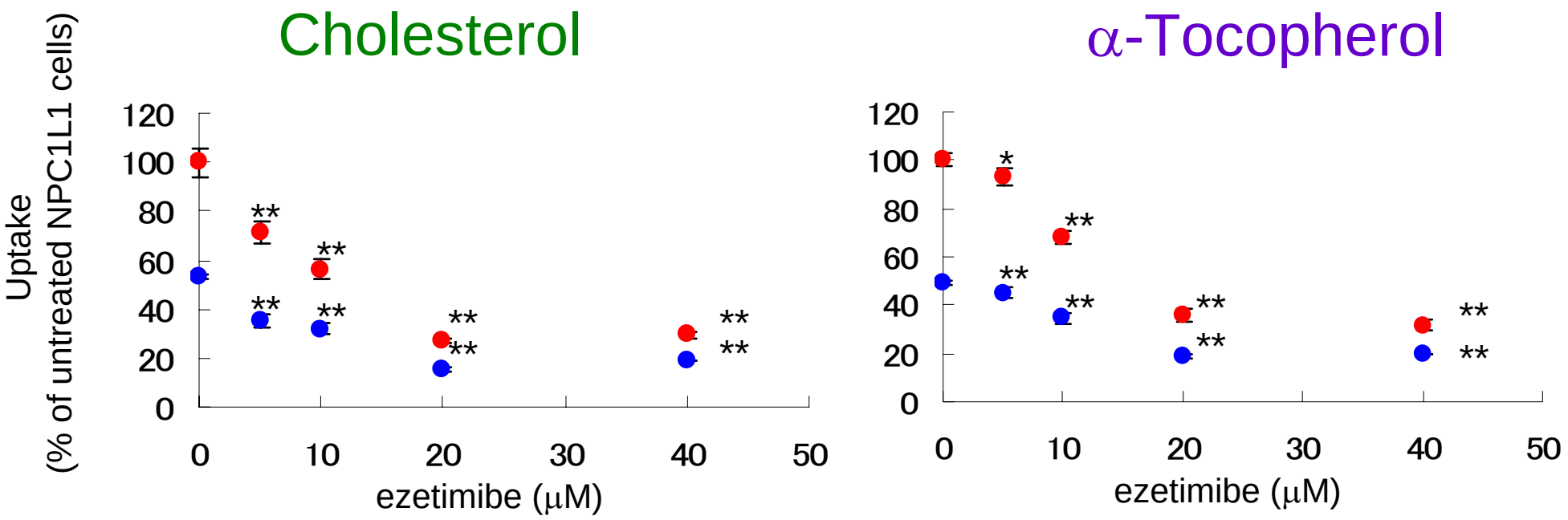
: Control cells

: NPC1L1 cells

**, p<0.01

Uptake (μl/mg protein) and time dependency of α-tocopherol uptake are similar to those of cholesterol uptake.

Inhibitory effect of ezetimibe on NPC1L1-mediated uptake



Calculated K_i value
Cholesterol : 10.6 ± 1.6 μM
α-Tocopherol : 17.1 ± 4.4 μM

: Control cells
: NPC1L1 cells

**, p<0.01
*, p<0.05

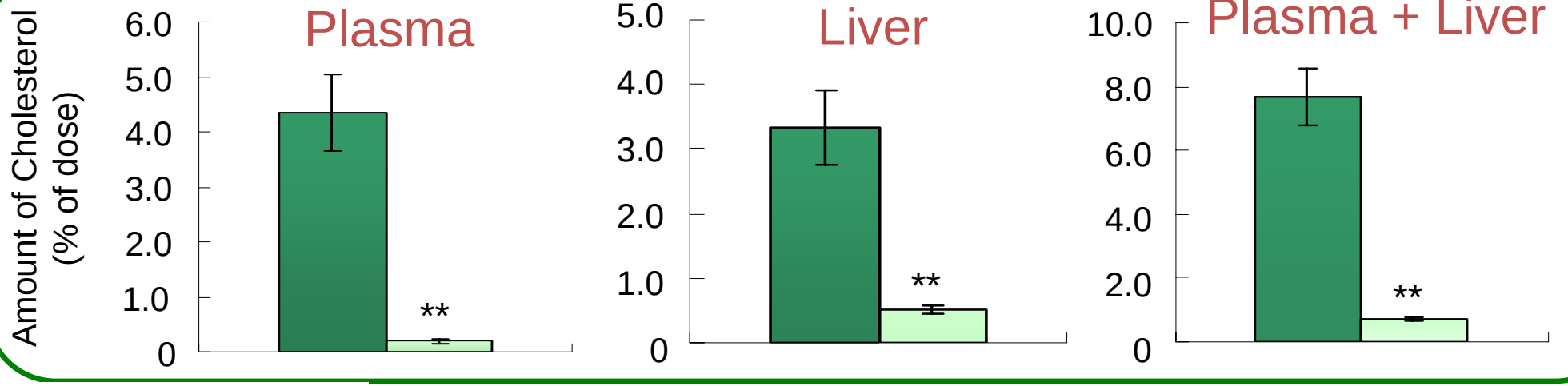
The inhibitory effect of ezetimibe on α-tocopherol uptake is nearly the same as that of cholesterol uptake.

NPC1L1 mediates α-tocopherol uptake in an ezetimibe-sensitive manner.

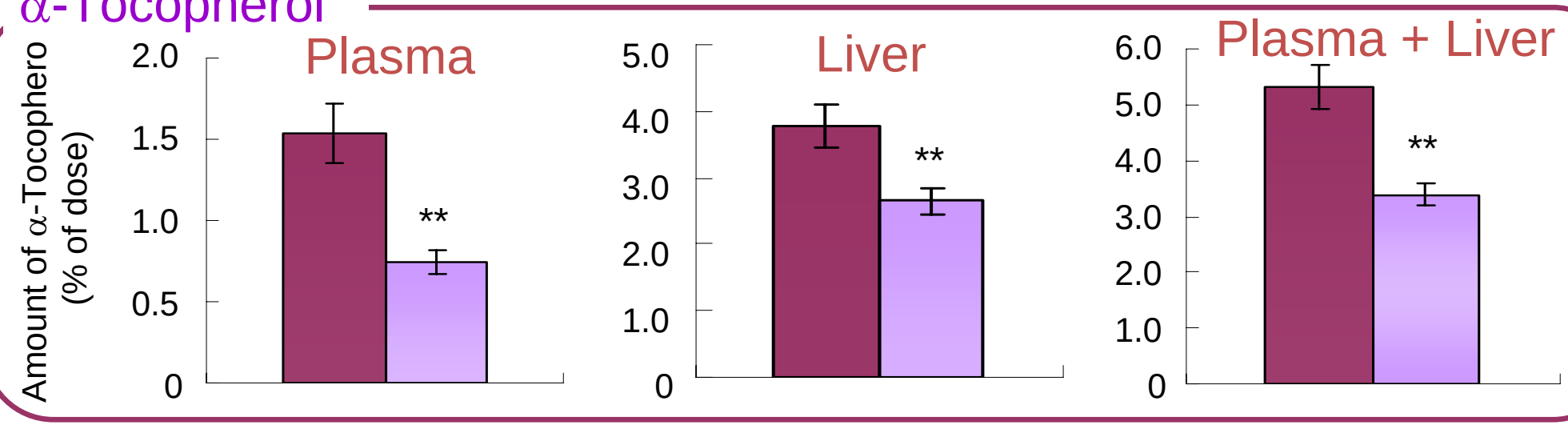
Inhibitory effect of ezetimibe *in vivo*

**: p<0.01

Cholesterol



α-Tocopherol

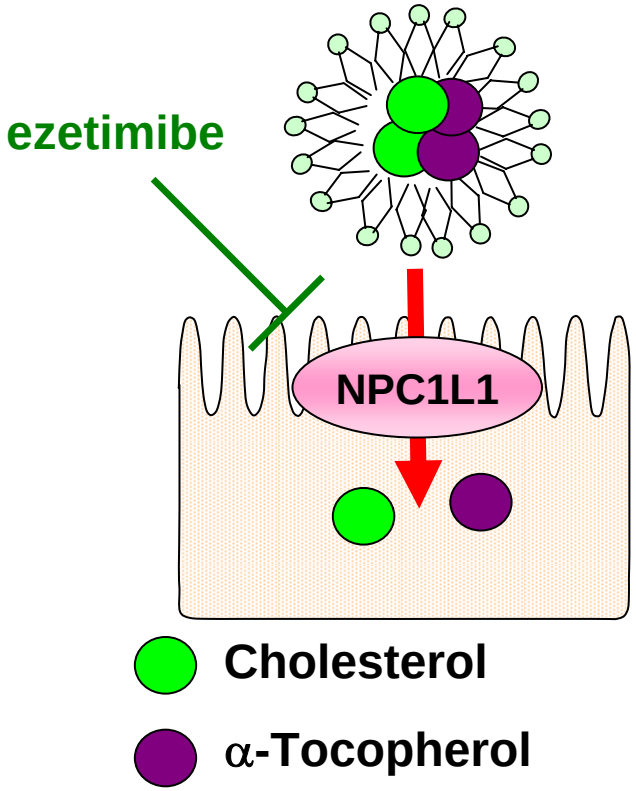


Ezetimibe inhibits intestinal absorption of α-tocopherol *in vivo*.

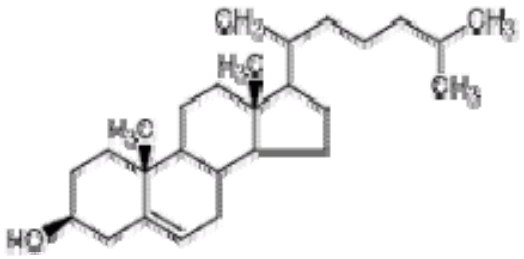
○ **NPC1L1 mediates the ezetimibe-sensitive uptake of all vitamin E tested in *in vitro* transport assays.**

○ **Results of *in vivo* absorption study suggested a physiologically significant role of NPC1L1-mediated vitamin E absorption.**

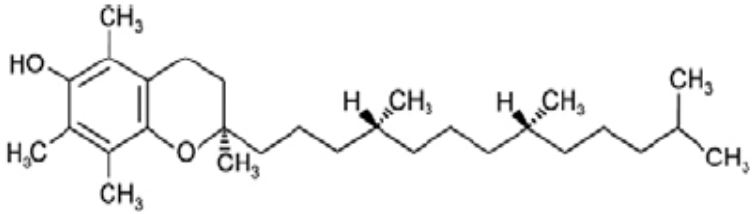
⇨ **The present data suggest that NPC1L1 is involved in the ezetimibe-sensitive absorption of vitamin E.**



Cholesterol



α-Tocopherol



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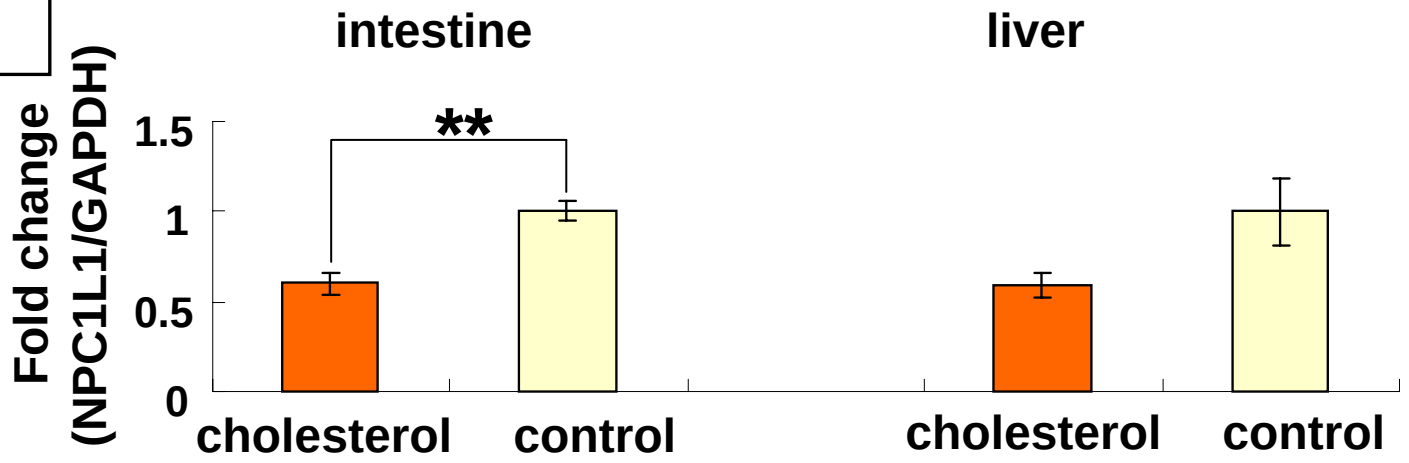
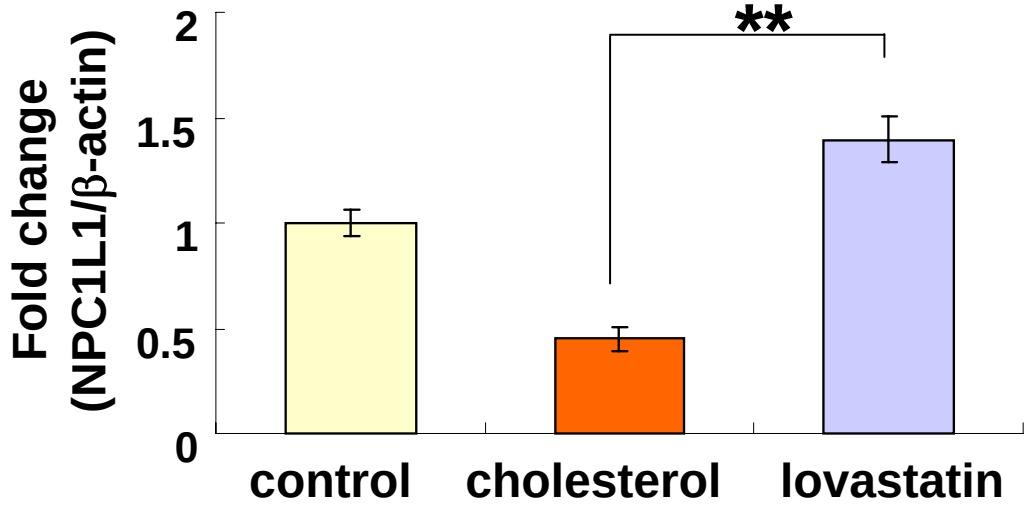
Effect of cholesterol on the expression of NPC1L1 mRNA *in vivo* and *in vitro*

mouse



HepG2

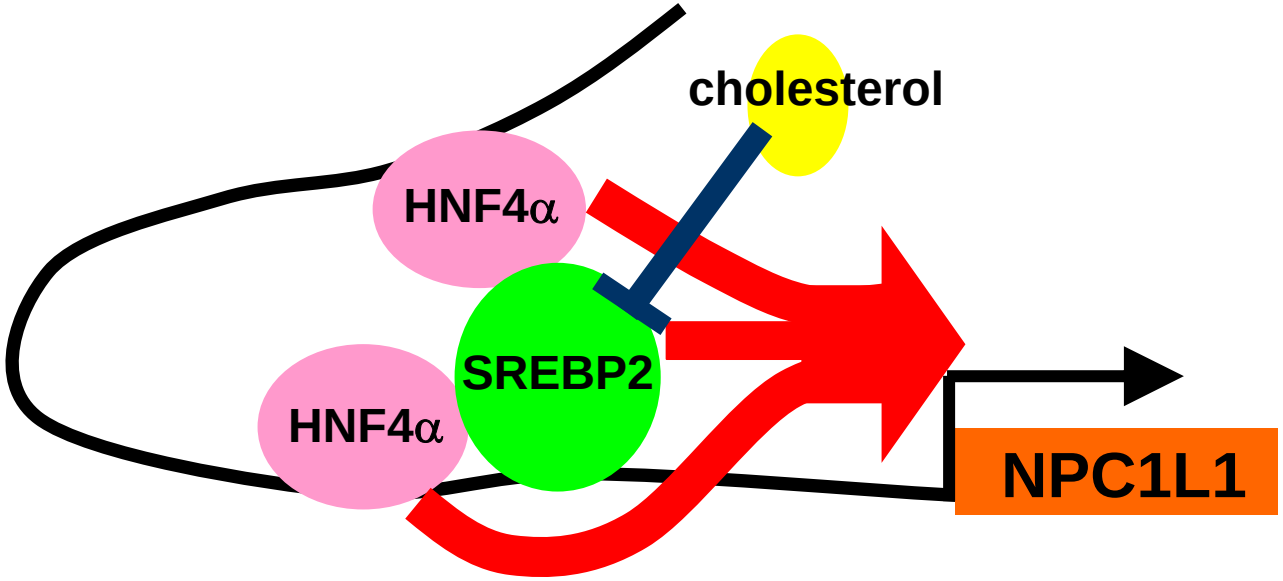


***P*<0.01

Cholesterol represses NPC1L1 expression *in vivo* and *in vitro*.

Regulatory mechanisms of NPC1L1 by HNF4α

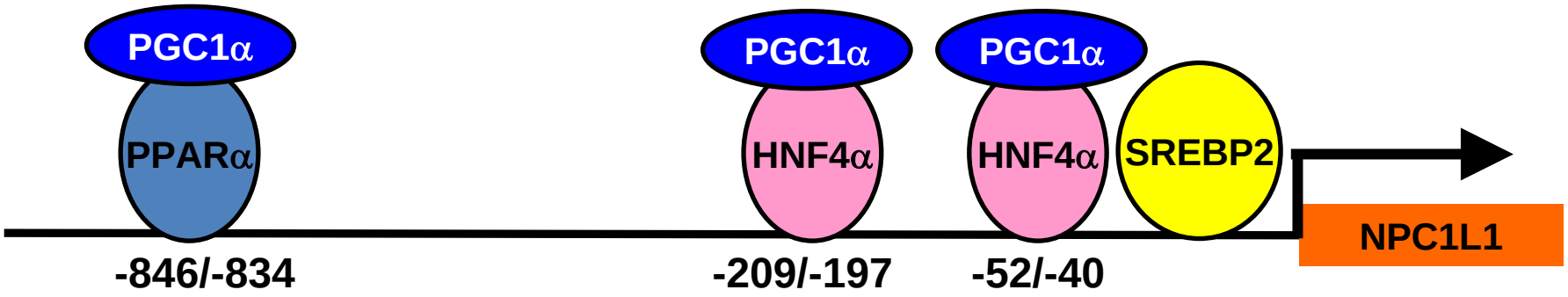
- HNF4α is a crucial modulator of hNPC1L1.
- HNF4α acts synergistically with SREBP2 on hNPC1L1 promoter.
- HNF4α is crucial for cholesterol-dependent expression of hNPC1L1.



Iwayanagi Y, Takada T, et al., *Pharm Res* (2008)

○PPARα positively regulates the expression of human NPC1L1 via a direct binding to the promoter region.

○PGC1α works as a coactivator of SREBP2/HNF4α and PPARα/RXRα on human NPC1L1 promoter.



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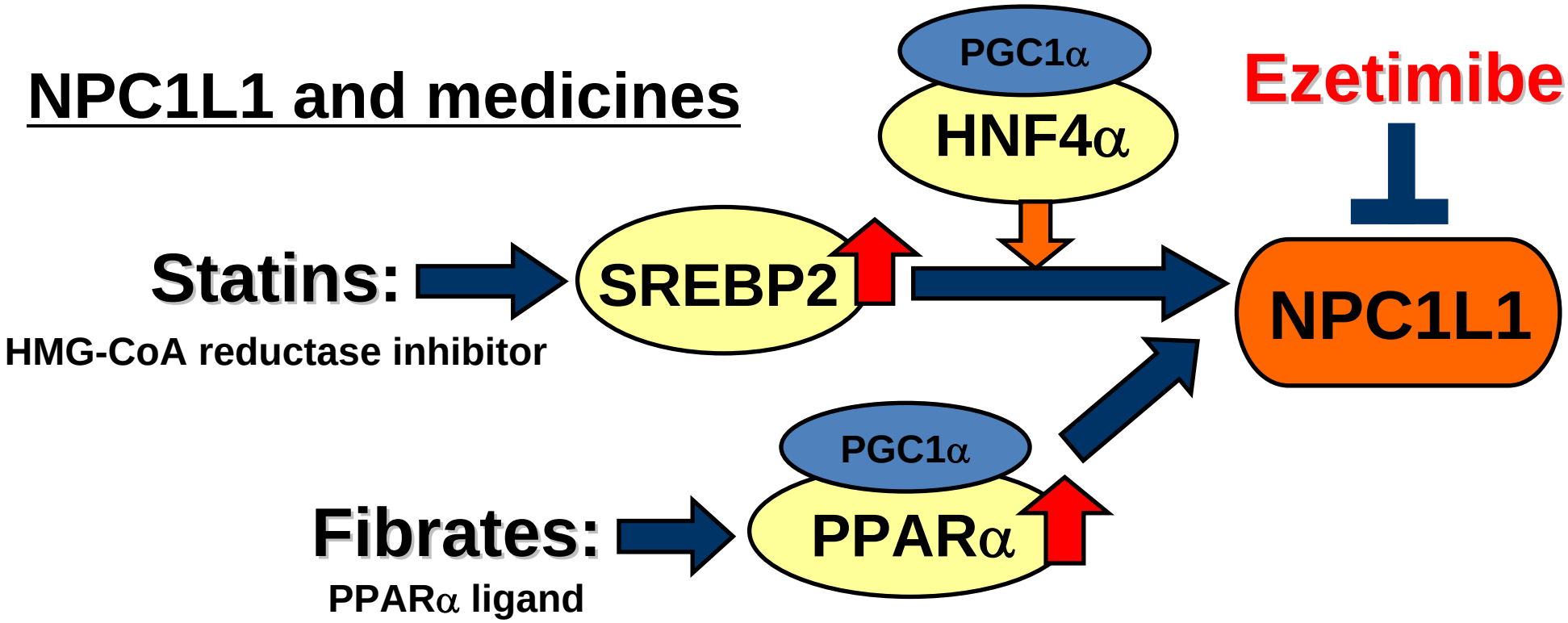
Clinical implications

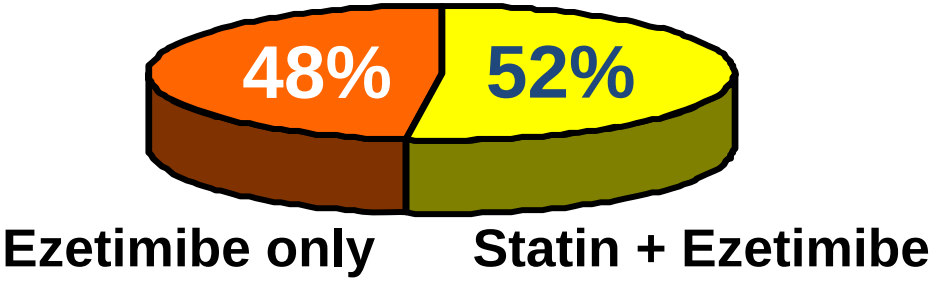
NPC1L1 and medicines



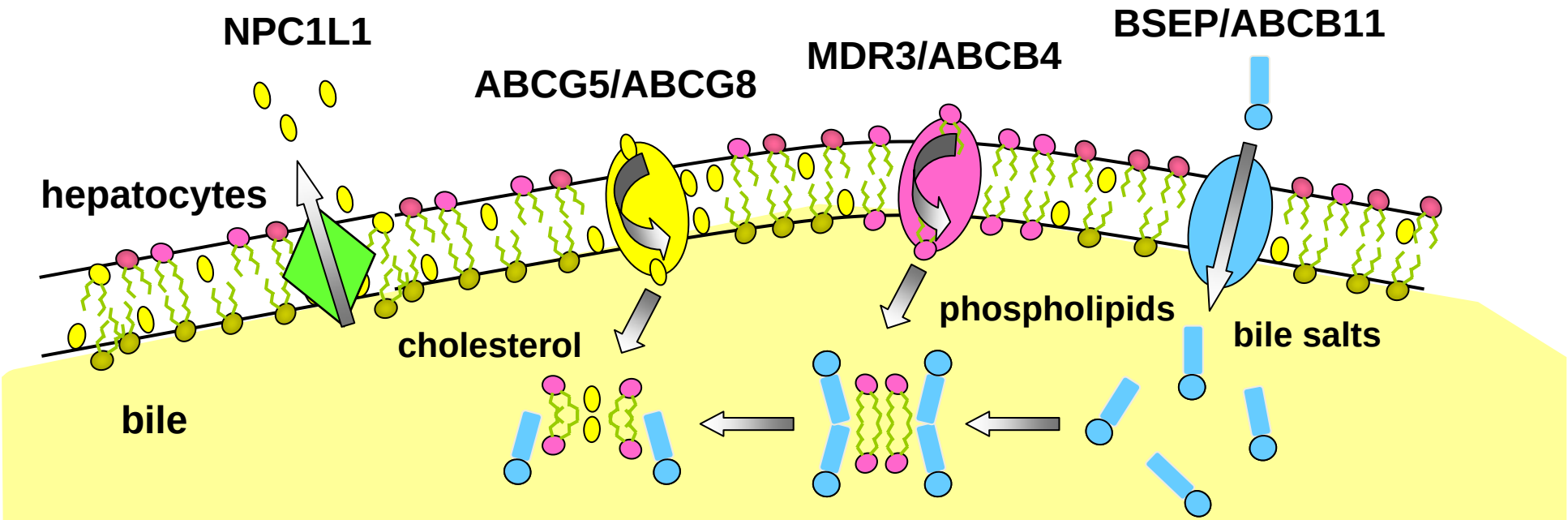
Statin + Ezetimibe

Fibrate + Ezetimibe

<Clinical use of Ezetimibe>



Bile lipids transporters on the canalicular membrane



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Hepatology (in press)

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Gastroenterology (2011)

Hepatol Res (2009)
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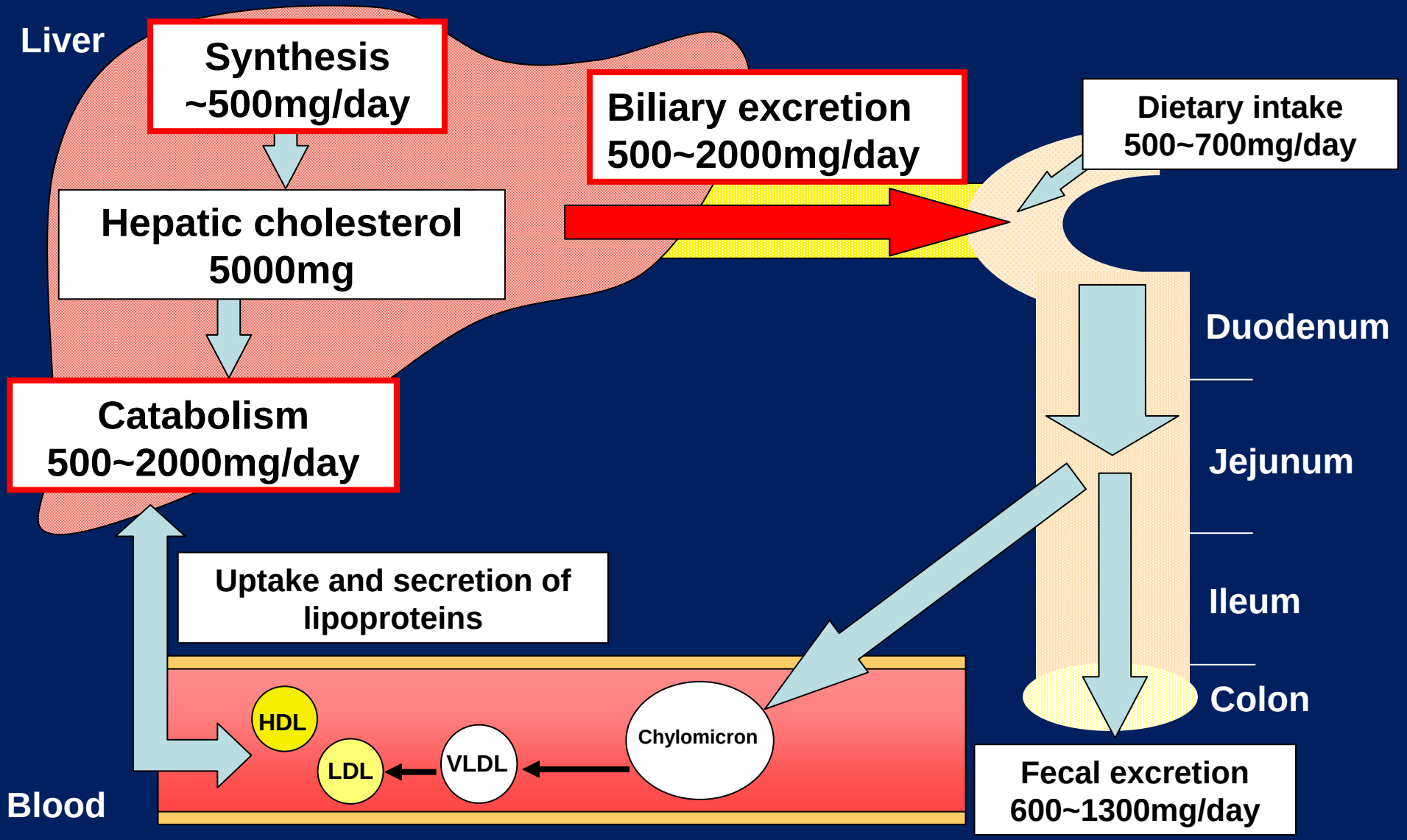
Hepatology (2005)
BBA (2005)
Mol Pharmacol (2011)

Cholesterol homeostasis is maintained by many steps



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