Review

Associations between Lifestyle-Related Diseases and Transporters Involved in Intestinal Absorption and Biliary Excretion of Cholesterol

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Westernization of dietary habits leads to an increase in lipid intake and is thought to be responsible for an increase in patients with dyslipidemia. It is a well-known fact that the impaired cholesterol homeostasis is closely related to the development of various lifestyle-related diseases such as fatty liver, diabetes, and gallstone as well as dyslipidemia leading to atherosclerosis and cardiovascular diseases such as heart attack and stroke. Therefore, appropriate management of cholesterol levels in the body is considered important in prevention and treatments of these lifestyle-related diseases and in addition, molecular mechanisms controlling plasma (and/or hepatic) cholesterol levels have been intensively studied. Due to its hydrophobicity, cholesterol was long believed to pass through cell membranes by passive diffusion. However, recent studies have identified a number of plasma membrane transporters that are responsible for the cellular uptake or efflux of cholesterol and involved in developments of lifestyle-related diseases. In this review, we focus on Niemann–Pick C1 Like 1 (NPC1L1) and a heterodimer of ATP-binding cassette transporter G5 and G8 (ABCG5/G8), both of which are responsible for intestinal cholesterol absorption and biliary cholesterol secretion, and discuss the relationship between these cholesterol transporters and lifestyle-related diseases. In addition, we also discuss the related uncertainties that need to be explored in future studies.

Key words cholesterol; plant sterol; vitamin; ezetimibe; intestinal absorption; biliary excretion

1. INTRODUCTION

The number of patients with metabolic disorders such as dyslipidemia, fatty liver, type II diabetes mellitus (T2DM), and gallstone diseases is increasing worldwide due to unhealthy lifestyle behaviors (i.e., lack of exercise, excessive eating of westernized diets, etc.). These lifestyle-related diseases are frequently caused by excess cholesterol (Fig. 1A) and, therefore, appropriate control of cholesterol levels in the body is thought to be important to prevent the lifestyle-related diseases.

Cholesterol levels in the body depend on the balance between de novo syntheses, catabolism, biliary excretion, and intestinal absorption from diets. Among these steps, biliary cholesterol excretion and intestinal cholesterol absorption are reported to be regulated by cholesterol transporters such as Niemann–Pick C1 Like 1 (NPC1L1)1-2) and a heterodimer of ATP-binding cassette transporter G5 and G8 (ABCG5/G8)3-4) (Fig. 2). Recent advances in genetic analyses such as genome-wide association study (GWAS) and studies with genetically

![Chemical Structures](image)
modified animals indicate that these cholesterol transporters might be involved in the development of several lifestyle-related diseases.

In this review, we provide an overview of recent findings about associations between these cholesterol transporters and lifestyle-related diseases, in addition to the pharmacological effect of ezetimibe (Fig. 1B), an NPC1L1 inhibitor clinically used to inhibit cholesterol (re)absorption, on the improvement of lifestyle-related diseases.

2. NPC1L1

2.1. NPC1L1 and Ezetimibe

In the intestinal lumen, dietary cholesterol is dissolved in the mixed micelles composed of bile acids and phospholipids. The micellar cholesterol was long believed to be taken up by intestinal epithelial cells via passive diffusion through the plasma membrane. However, the discovery of ezetimibe, a potent inhibitor of intestinal cholesterol absorption, raised the possibility that the cholesterol absorption occurs not only via passive diffusion but also via protein-mediated processes (mechanisms). In the 1990’s when ezetimibe was developed, the molecular target of this drug was not uncovered. However, in 2004, Altmann et al. first reported that NPC1L1 is a key molecule to ezetimibe-sensitive intestinal cholesterol absorption. 1)

NPC1L1 was originally identified as a homologue of Niemann–Pick C1 (NPC1) protein. 5) NPC1 is involved in the intracellular cholesterol trafficking from lysosome to other organelles such as plasma membrane and endoplasmic reticulum (ER). 6,7) In humans, dysfunction of NPC1 protein causes Niemann–Pick disease type C (NPC disease), a genetic disorder characterized by lysosomal cholesterol accumulation resulting in neurodegeneration and premature death. 8,9) Unlike NPC1, physiological functions of NPC1L1 remained unclear for a

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**Biography**

Graduated from Faculty of Pharmaceutical Sciences, The University of Tokyo (1999), Research Fellow for Young Scientists from JSPS (DC1) (2001–2004), Graduated from Graduate School of Pharmaceutical Sciences, the University of Tokyo (2004). Research Assistant (2004–2007), Assistant Professor (2007–2012), Associate Professor (2012–present) and Vice Director of Department of Pharmacy (2013–present) at the University of Tokyo Hospital. He spent 6 years from undergraduate to Ph.D. at Professor Yuichi Sugiyama’s laboratory. After he obtained Ph.D., he has worked with Professor/Director Hiroshi Suzuki. His research focuses on the pharmacokinetics of physiological substrates of transporters, especially substrates involved in lifestyle-related diseases, such as cholesterol for dyslipidemia and urate for hyperuricemia and gout. His Mottoes are “as simple as possible” and “positive thinking.”
long time probably because NPC1L1 knockout (KO) mice appeared healthy without severe phenotypes. However, in the process of seeking a molecular target of ezetimibe, Altmann et al. found that intestinal cholesterol absorption in NPC1L1 KO mice was reduced to approximately 30% of that in wild-type (WT) mice and the degree of this reduction was almost the same as that observed in ezetimibe-treated WT mice.  

Furthermore, they revealed that NPC1L1 is highly expressed on the brush border membrane in the duodenum and jejunum, where cholesterol absorption primarily occurs. From these results and in vitro observations that ezetimibe-glucuronide, an active metabolite of ezetimibe, binds to NPC1L1 protein, NPC1L1 is now thought to be a central player in intestinal cholesterol absorption and a molecular target of ezetimibe.

### 2.2. NPC1L1 and Dyslipidemia

Gene mutation analyses in humans demonstrated that heterozygous carriers of NPC1L1 inactivating mutations have a mean cholesterol level that was lower than that in non-carriers, and in addition, coronary heart disease risk in carriers of the NPC1L1 mutation was 54% lower than that in non-carriers.  

Consistent with these results, several clinical studies demonstrated that ezetimibe administration reduced plasma cholesterol levels (low-density lipoprotein (LDL)-cholesterol levels in particular). In addition, a recent clinical trial demonstrated that ezetimibe administration reduced the rate of cardiovascular events and the extent of this reduction was similar to those observed by treatment with statins, hydroxymethylglutaryl-CoA (HMG-CoA) reductase inhibitors that suppress de novo cholesterol synthesis, when adjusted for the cholesterol lowering intensity. All of these data indicate that inhibition of NPC1L1-mediated intestinal cholesterol absorption is effective to improve dyslipidemia and thus, to prevent atherosclerotic diseases.

Previous studies revealed that the mRNA level of human NPC1L1 is positively regulated by sterol-regulatory elemental binding protein 2 (SREBP2), a transcriptional factor expressed in the intestine and liver. Since SREBP2 is activated when intracellular cholesterol level is decreased, NPC1L1 expression increases in response to cholesterol deficiency via the SREBP2-mediated transcription. In addition to the expression level, cellular localization of NPC1L1 protein is also regulated by cholesterol. Several in vitro studies demonstrated that NPC1L1 translocates from intracellular vesicles to the plasma membrane in response to the cholesterol depletion by methyl-β-cyclodextrin, facilitating the NPC1L1-mediated cholesterol uptake.  

Considering the NPC1L1 function as a cholesterol importer, these cholesterol-sensitive regulations of NPC1L1 expression and localization are rational from the viewpoint of cholesterol homeostasis. However, such a homeostatic response of NPC1L1 often makes it difficult to treat dyslipidemia. For instance, patients administered statins frequently exhibit increased expression of NPC1L1 in the intestine and, therefore, their cholesterol absorption is elevated, which attenuates the cholesterol-lowering effect of statins. Thus, combination therapies with statins and ezetimibe have been recommended in the treatment for dyslipidemia. Indeed, several clinical studies revealed that co-administration of statins and ezetimibe could reduce plasma (LDL-)cholesterol more effectively compared with either monotherapy. In addition, based on these results, combination drugs that contain fixed-doses of ezetimibe and statin (simvastatin, rosvastatin or atorvastatin) have been developed and used clinically.

These facts indicate the importance of taking account of cholesterol homeostasis in pharmacotherapy for dyslipidemia.

### 2.3. NPC1L1 and Fatty Liver Diseases

Non-alcoholic fatty liver disease (NAFLD) is a progressive disorder that is caused by an accumulation of lipids such as triglycerides and cholesterol in hepatocytes without heavy alcoholic consumption. Non-alcoholic steatohepatitis (NASH) is the most extreme form of NAFLD, which is characterized by steatosis, necroinflammation, and cytopathic changes, causing liver cirrhosis. Recently, the number of patients with NAFLD and/or NASH (NAFLD/NASH) has dramatically increased worldwide in association with the food satiation and the westernization of eating habits. Several in vivo studies demonstrated that high cholesterol diets could induce hepatic lipids accumulation, resulting in substantial inflammation and fibrosis, and exaggerate NASH in mice.  

In addition, it has been revealed that NPC1L1 KO mice are tolerant to diet-induced development of NAFLD/NASH (27,28) Consistent with the results in animal models, several clinical studies demonstrated that ezetimibe attenuates hepatic steatosis and improves serum liver enzymes such as ALT, aspartate aminotransferase (AST), and γ-glutamyltransferase (γ-GTP), in NAFLD/NASH patients. All of these data suggest that NPC1L1-mediated intestinal cholesterol absorption is involved in the pathogenesis of NAFLD/NASH.

As the mechanism of NAFLD/NASH progression, “two hits” model has been proposed for a long time. The “first hit” is the deposition of lipids in hepatocytes. The “second hit” consists of a variety of cellular stresses, such as oxidative stress, ER stress, and apoptosis, that induce liver inflammation. One of proposed mechanisms underlying beneficial effects of ezetimibe on NAFLD/NASH progression is inhibition of lipogenesis in the liver. Decreased cholesterol absorption causes reduction in hepatic cholesterol amounts and concomitantly suppresses cholesterol-dependent activation of liver X receptor α (LXRA/NR1H3), subsequently decreasing hepatic expressions of transcriptional factors involved in the lipogenesis, such as sterol regulatory element-binding protein-1c and carbohydrate response element-binding protein. Thus, inhibition of cholesterol absorption would lead to prevent the “first hit”: excess accumulation of lipids in the liver. In addition, Lee et al. recently demonstrated that ezetimibe has potential to activate nuclear factor erythroid 2-related factor 2 (Nrf2), a master transcriptional factor whose target genes include antioxidant proteins and detoxification enzymes. They revealed that the ezetimibe-mediated activation of Nrf2 could protect liver from oxidative injury caused by diet-induced NASH in mice. These data indicate that ezetimibe may inhibit not only the “first hit,” but also the “second hit” to prevent the progression of NAFLD/NASH. Further studies with NPC1L1 KO mice will be necessary to elucidate whether the ezetimibe-mediated Nrf2 activation is related to the inhibition of NPC1L1 or not.

Unlike rodents, in which NPC1L1 is predominantly ex-
pressed in the intestine, humans highly express NPC1L1 in the liver as well as in the intestine. Therefore, hepatic NPC1L1 in addition to intestinal NPC1L1 should be considered when discussing NAFLD/NASH progression in humans. So far, several studies were conducted to elucidate physiological function of hepatic NPC1L1. In vivo studies with liver-specific human NPC1L1 transgenic (TG) mice revealed that hepatic NPC1L1 is localized on the bile canalicular membrane and biliary cholesterol concentration in NPC1L1 TG mice is much less than that in WT mice, suggesting that hepatic NPC1L1 is involved in the cholesterol reuptake from bile to hepatocytes. Besides such a role as a cholesterol importer on the bile canalicular membrane, we found that hepatic NPC1L1 at ER down-regulates the protein expression and secretion of Niemann–Pick C2 (NPC2), a cholesterol-binding protein whose dysfunction causes NPC disease (Fig. 3), in an ezetimibe-insensitive manner. Previous studies demonstrated that in mice and humans, NPC2 is expressed in the liver and secreted into bile. Cellular NPC2 is known to be involved in the intracellular cholesterol trafficking from lysosome to other organelles in cooperation with NPC1. On the other hand, regarding secreted NPC2, although its physiological function was unknown for a long time, we recently demonstrated that biliary NPC2 has ability to stimulate ABCG5/G8-mediated cholesterol efflux from hepatocytes to bile (Fig. 3). Both of these NPC2 functions would contribute to suppress the hepatic cholesterol accumulation. From these facts, it is suggested that hepatic NPC1L1 may facilitate cholesterol accumulation in the liver by reabsorbing cholesterol from bile to hepatocytes and simultaneously by down-regulating the hepatic expression and biliary secretion of NPC2. Considering the species difference in hepatic expression of NPC1L1, it is possible that humans are more likely to develop NAFLD/NASH than rodents. In this viewpoint, pathological analyses of NPC1L1 TG mice as a human model might be preferable and would provide new insights to the pathogenesis of NAFLD/NASH in humans.

2.4. NPC1L1 and T2DM

T2DM is one of common metabolic disorders characterized by high blood glucose levels and insulin resistance. It is known that hepatic cholesterol accumulation induces ER stress and generates reactive oxygen species in the liver, both of which lead to hepatic insulin resistance through inactivation of Akt, resulting in T2DM. Because of such a close association between cholesterol accumulation and insulin resistance in the liver, it is plausible that NPC1L1 is to some extent involved in the T2DM progression. Consistently, several studies have demonstrated that ezetimibe therapy can improve insulin sensitivity and glucose intolerance in rodents and humans, suggesting that NPC1L1-mediated cholesterol (re)absorption is a pathophysiologically important factor for the development of T2DM. Considering a number of clinical observations that statin therapy modestly increased the risk of diabetes onset, although this increase appears to be small and outweighed by the benefits of statins on cardiovascular disease prevention, ezetimibe might be superior to statins at least in terms of T2DM prevention.

Interestingly, a recent study with T2DM model rats (Otsuka Long-Evans Tokushima Fatty rats), which are characterized by hyperphagia-induced obesity and T2DM due to spontaneous lack of cholecystokinin 1 receptors that inhibit food intake action, revealed that chronic administration of ezetimibe increased serum glucagon-like peptide-1 (GLP-1) and improved hyperglycemia. GLP-1 is an incretin hormone secreted from intestinal epithelial L cells, which exerts anti-diabetic actions by increasing insulin secretion from pancreatic β-cells and by inhibiting glucagon release. Although a subsequent study revealed that the activation of mitogen-activated protein/extracellular signal-regulated kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathway is a key
in the ezetimibe-related GLP-1 secretion, it has not yet been uncovered whether NPC1L1 is directly involved in the GLP-1 secretion or not. In vivo studies with NPC1L1 KO mice are essential to reveal the relationship between NPC1L1 and GLP-1.

In addition to the effects of NPC1L1 and ezetimibe on T2DM progression as described above, it is also known that hyperglycemia affects NPC1L1-mediated cholesterol absorption. Indeed, it was reported that mRNA expression of NPC1L1 in the intestine was elevated in diabetic patients, which may result in an increase in serum cholesterol levels. Regarding the regulatory mechanism of NPC1L1 expression, we demonstrated that, in addition to SREBP2, several transcriptional factors such as hepatocyte nuclear factor 4α (HNF4α/NR2A1), peroxisome proliferator-activated receptor α (PPARα/NR1C1), and PPARγ coactivator 1α (PGC1α) positively regulate the mRNA expression of NPC1L1. Taken together with facts that both HNF4α and PPARα are involved in the pathogenesis of diabetes and that intestinal expression of PGC1α is increased under diabetic conditions, our findings indicate that these transcriptional factors may play important roles in the regulation of NPC1L1 expression under diabetic conditions and at least partly account for clinical associations between progressions of T2DM and other cholesterol-related diseases such as dyslipidemia and NAFLD/NASH.

2.5. Physiological Substrates of NPC1L1 Other than Sterols and Potential Risks Associated with Ezetimibe Therapy

Physiological substrates of NPC1L1 were believed to be only sterols such as cholesterol and plant sterols. However, our recent studies demonstrated that NPC1L1 has ability to transport several fat-soluble nutrients such as vitamin E (α-tocopherol and other isomers) and vitamin K (phyloquinone: a major component of dietary vitamin K) (Fig. 1D). In addition, we also revealed that ezetimibe can inhibit the intestinal absorption of these fat-soluble vitamins in mice and rats. Our findings suggest that intestinal absorption of vitamin E and vitamin K is mediated by ezetimibe-sensitive NPC1L1-dependent pathway (Fig. 2). Vitamin E acts as an antioxidant, potentially inhibiting the occurrence of cardiovascular events. Vitamin K inhibits the development of arteriosclerosis by activating the matrix Gla protein, which suppresses vascular calcification. These facts support the assumption that, besides its function as a cholesterol importer, NPC1L1 plays a pivotal role in the absorption of vitamin E and vitamin K to prevent the harmful effects of excess cholesterol in the body.

Although ezetimibe inhibits vitamin E absorption by approximately 35% in vivo, a clinical study has reported that serum concentrations of vitamin E were not significantly reduced after a 12-week administration of ezetimibe. Because α-tocopherol transfer protein (α-TTP), a cytosolic protein expressed in the liver, plays a key role in maintaining vitamin E homeostasis by regulating hepatic storage and trafficking of α-tocopherol, the partial inhibition of vitamin E absorption by ezetimibe may not immediately cause vitamin E deficiency in humans. However, considering that humans cannot synthesize vitamin E and that it is therefore necessary to ingest this nutrient from diets, the ezetimibe-mediated malabsorption of vitamin E may be taken into consideration as a potential risk for vitamin E insufficiency.

With respect to ezetimibe-related malabsorption of vitamin K, a drug interaction between ezetimibe and warfarin (Fig. 1E) should be noted. Warfarin is an anticoagulant drug clinically used for thrombosis. It is known that clotting factors are activated in the liver through the cyclic conversion of hepatic vitamin K (vitamin K cycle) and that warfarin exerts its anticoagulant activity by inhibiting the vitamin K cycle. Interestingly, it has been reported that anticoagulant activity of warfarin is enhanced by combination with ezetimibe. Figure 4A shows typical cases of the ezetimibe–warfarin interaction found by our retrospective survey of clinical records at the University of Tokyo Hospital. Although the mechanism of this drug–drug interaction was not clear, we recently demonstrated that ezetimibe-related vitamin K₃ malabsorption causes reduction in hepatic vitamin K₃ amounts, resulting in an unintentional enhancement of anticoagulant activity in almost all (more than 85%) patients taking both warfarin and ezetimibe simultaneously (Fig. 4B). It should be noted, however, that such an unintentional effect of ezetimibe on blood coagulation was hardly observed in patients not taking warfarin, which was consistent with pharmacological studies with rats. In the liver, the recycling of vitamin K via the vitamin K cycle regulates hepatic vitamin K robustness. Therefore if the vitamin K cycle is functional, inhibition of intestinal vitamin K₃ absorption should not directly affect blood coagulation. However, the inhibition of the vitamin K cycle by warfarin should disrupt the control of the vitamin K robustness, which increases response to the supply of vitamin K from the intestine (Fig. 4B). These facts indicate that ezetimibe-related vitamin K₃ malabsorption is a clinically significant issue in patients taking warfarin and thus, prothrombin time international normalized ratio (PT-INR), a biomarker for anticoagulant activity, should be carefully monitored when these patients start ezetimibe therapy.

Given that chemical structures of cholesterol (plant sterols), vitamin E, and vitamin K are largely different (Fig. 1), the substrate specificity of NPC1L1 may be relatively broad. Since some of fat-soluble nutrients and clinically used drugs in addition to vitamin E and vitamin K₃ have been reported to exhibit similar in vivo behaviors to cholesterol, it is possible that such compounds may also be absorbed via NPC1L1-mediated pathway in the intestine. In order to fully understand physiological functions of NPC1L1 and to more accurately predict pharmacological effects of ezetimibe, a comprehensive understanding of NPC1L1 substrates would be necessary.

3. ABCG5/G8

3.1. ABCG5/G8 and Sitosterolemia

ABC4G5 and ABCG8 are ABC transporters identified as causative genes of sitosterolemia with an abnormally high levels of plant sterols in the blood. Both of them are six-transmembrane proteins with one ATP-binding site and are localized on the plasma membrane as heterodimers. In mammals, ABCG5/G8 is highly expressed on the brush border membrane in the intestine and on the bile canalicular membrane in the liver and eliminates cholesterol and plant sterols to the intestinal lumen and bile. Consistent with its role in preventing excess cholesterol accumulation, mRNA expression of ABCG5/G8 increases in the liver and the intestine in response to cholesterol feeding. Since this response was lost in mice lacking both LXRα and LXRβ/NR1H2, LXR-signaling pathway should be
involved in the cholesterol-dependent regulation of ABCG5/G8 expression. In addition, treatment with cholic acid (Fig. 1F), a major primary bile acid produced from cholesterol, was reported to increase hepatic expressions of ABCG5/G8 via the farnesoid X receptor (FXR/NR1H4)-dependent mechanism, resulting in increased biliary cholesterol secretion. These observations suggest that the expression level of ABCG5/G8 is positively regulated by cholesterol and cholesterol metabolites.

Pathological characteristics of sitosterolemia include xanthomas and premature coronary artery disease due to the accumulation of sterols (plant sterols in particular) in the body. In addition, an abnormality in steroid hormone production by the accumulation of sterols in the adrenal grand has been reported, indicating that adequate sterol elimination by ABCG5/G8 is important for the appropriate syntheses of steroid hormones and their physiological actions. Furthermore, stomatocytosis, hemolytic anemia, thrombocytopenia with very large platelets, splenomegaly, and abnormal bleeding was reported in patients with sitosterolemia. Since ABCG5/G8 is little expressed in red blood cells and platelets, the abnormal morphologies and functions of the blood cells observed in sitosterolemia seems to be secondary to the accumulation of plant sterols in the blood. Indeed, Kanaji et al. demonstrated that incorporation of plant sterols into the platelet membrane causes hyperactivation of platelet, leading to bleeding abnormalities and macrothrombocytopenia. These pathological characteristics of sitosterolemia reveal the potential toxicities of plant sterols and also indicate the importance of physiological function of ABCG5/G8 to eliminate such toxic sterols.

Pharmacotherapies with ezetimibe are currently considered as first choice therapy for sitosterolemia. Consistent with the in vivo observation that NPC1L1 KO mice showed a reduced intestinal absorption of β-sitosterol (one of major plant sterols) (Fig. 1A) and in vitro findings that NPC1L1

![Drug Interaction between Ezetimibe and Warfarin](image-url)
has an ezetimibe-sensitive transport activity for β-sitosterol, ezetimibe can reduce intestinal absorption of plant sterols in vivo. So far, several clinical studies demonstrated that long-term treatment with ezetimibe was safe, tolerable, and effective in reducing plasma plant sterol levels in patients with sitosterolemia, resulting in regression of xanthomas, improvement of cardiovascular function, and decrease in the risk of bleeding.

### 3.2. ABCG5/G8 and Gallstone
Although ABCG5/G8 KO mice showed increased liver weight, hepatic lipids accumulation, higher plasma ALT, and increased fasting glucose levels compared with WT mice, there is little information on liver histology and glucose metabolism in patients with sitosterolemia except for one patient with chronic active hepatitis and signs of cirrhosis. Meanwhile, associations of cholesterol gallstone disease with mutations of ABCG5/G8 have been revealed in humans by a number of gene mutation analyses and GWAS.

The number of patients with cholesterol gallstone disease as well as other metabolic disorders including dyslipidemia, NAFLD/NASH, and T2DM (insulin resistance) is increasing worldwide. In addition to environmental factors such as westernization of diets, genetic factors for the risk of gallstone disease have been clarified. For instance, hereditary contribution of cholesterol 7a-hydroxylase and apolipoprotein E and B genes to gallstone formation were reported. In addition, as described above, based on the fact that hypersecretion of cholesterol into bile has been identified as the primary pathogenic event in gallstone formation, gene mutations and single nucleotide polymorphisms (SNPs) of ABCG5 and ABCG8 have attracted considerable attention. To date, several SNPs of ABCG5 and ABCG8 were reported to be associated with cholesterol gallstone disease. A recent meta-analysis demonstrated that among these SNPs, ABCG8 D19H (rs11887534) in particular exhibit the strong association with gallstone diseases. Regarding this SNP, it has also been reported that carriers of the mutant variant (ABCG8 19H) exhibit decreased intestinal cholesterol absorption. Considering the physiological function of ABCG5/G8 as a sterol exporter, these findings indicate that the mutant variant might have a more efficient cholesterol efflux activity compared with the wild-type variant (ABCG8 19D). In this case, however, it is seemingly contradictory that the mutant carriers with low cholesterol absorption tend to develop gallstones. Similarly, recent clinical observations that ezetimibe can prevent cholesterol gallstone disease are also apparently inconsistent with the result showing that ezetimibe can inhibit not only dietary cholesterol absorption but also biliary cholesterol reabsorption by hepatic NPC1L1.2)

Such apparent “contradiction” might be accountable as follows. The amount of biliary cholesterol secretion, which is a major factor involved in cholesterol gallstone formation, would be determined by multiplying the cholesterol export activity from the liver to bile and the hepatic cholesterol amount controlled to some extent by intestinal cholesterol absorption as described in the previous section 2.3. Thus, in the case where physiological activity (or contribution) of the cholesterol transporters is different between in the liver and intestine as following: hepatic ABCG5/G8 > intestinal ABCG5/G8, meanwhile hepatic NPC1L1 < intestinal NPC1L1, the above “contradiction” can be explainable. Indeed, it has been reported that mRNA expression of ABCG5 in the liver is higher than that in the intestine in humans. Further studies using liver- or intestine-specific knockout (or transgenic) mice are necessary to elucidate such a balance of cholesterol transport activity of ABCG5/G8 and NPC1L1 in each tissue and to clarify the exact contribution of these transporters in the biliary cholesterol secretion and intestinal cholesterol absorption.

Regarding the regulation of biliary cholesterol secretion, functional association among ABCG5/G8, NPC2, and NPC1L1 should also be considered (Fig. 3), as described in the section 2.3. Our finding that biliary NPC2 stimulates ABCG5/G8-mediated cholesterol efflux together with the recent observation that secreted NPC2 can facilitate cholesterol nucleation and crystallization, suggests the possibility that biliary NPC2 would promote gallstone formation by stimulating biliary secretion, nucleation, and crystallization of cholesterol. Meanwhile, hepatic NPC1L1 may contribute to prevent gallstone formation through the down-regulation of biliary NPC2 in addition to its direct role in cholesterol reuptake to hepatocytes (Fig. 3). Given that humans are more likely to form gallstones than rodents due to differences in biliary bile acids composition, the abundant expression of hepatic NPC1L1 in humans but not in rodents is rational to prevent gallstone formation in humans.

### 4. CONCLUSION
Cholesterol is involved in several physiological and/or pathophysiological processes such as lipogenesis and insulin resistance through the regulation of several transcriptional factors and signaling pathways. Therefore, maintaining adequate concentrations of cholesterol in the blood and tissues is important in preventing and improving a variety of lifestyle-related diseases. When we consider that the liver and small intestine are main tissues controlling cholesterol homeostasis, it makes sense that cholesterol transporters such as NPC1L1 and ABCG5/G8 express abundantly and function effectively in these tissues to regulate cholesterol levels in the body. Physiological functions of each cholesterol transporter have been revealed by in vitro analyses and in vivo studies with gene KO and/or TG mice. However, recent findings have indicated that these cholesterol transporters cooperatively regulate their expression levels and transport activities with each other by cholesterol-dependent transcriptional signals and/or cholesterol-related molecules such as NPC2. Thus, in order to understand the whole picture of regulatory mechanism for cholesterol homeostasis, it will be necessary to clarify the mutual regulation (interaction) between these transporters under both physiological and pathophysiological conditions. Further studies from this point of view would provide novel insights to the development of effective therapies for lifestyle-related diseases.

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