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RESEARCH PAPER

Human NPC1L1 Expression is Positively Regulated by PPARα

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ABSTRACT

Purpose Niemann-Pick C1-like 1 (NPC1L1), a pharmacological target of ezetimibe, is responsible for cholesterol absorption in enterocytes and hepatocytes. In the present study, the involvement of peroxisome proliferator-activated receptor α (PPARα) and its cofactor, PPARγ coactivator 1α (PGC1α) in the transcriptional regulation of human NPC1L1 was analyzed.

Methods Reporter gene assays and electrophoretic mobility shift assays (EMSAs) were performed with the 5′-flanking region of the human NPC1L1 gene and the effect of siPPARα was examined.

Results PPARα-mediated transactivation was observed with human NPC1L1 promoter constructs. Detailed analyses using deletion- and mutated-promoter constructs revealed the presence of a functional PPARα-response element (PPRE) upstream of the human NPC1L1 gene (−846/−834), a direct binding of PPARα and RXRα to which was confirmed by EMSAs. Moreover, PPARα-specific knockdown resulted in a significant decrease in the endogenous expression of NPC1L1 mRNA and protein in human-derived HepG2 cells. Furthermore, cotransfection of PGC1α stimulated the SREBP2/HNF4α- and PPARα/RXRα-mediated activation of the human NPC1L1 promoter.

Conclusions We found that PPARα positively regulates human NPC1L1 transcription via direct binding to a PPRE. Additionally, PGC1α stimulates the SREBP2/HNF4α- and PPARα/RXRα-mediated transactivation of human NPC1L1. These findings may provide new insights into the close relationship of glucose, fatty acids and cholesterol homeostasis.

KEY WORDS cholesterol · ezetimibe · fenofibrate · fibrates · transporter

ABBREVIATIONS

BSA	bovine serum albumin
DMEM	Dulbecco's Modified Eagle Medium
DR-1	direct repeat-1
EMSA	electrophoretic mobility shift assay
HNF4α	hepatocyte nuclear factor 4α
I-FABP	intestinal-fatty acid binding protein
NPC1L1	Niemann-Pick C1-like 1
PGC1α	PPARγ coactivator 1α
PPAR	peroxisome proliferator-activated receptor
PPRE	PPARα-response element
RXR	retinoid X receptor
SDS	sodium dodecyl sulfate
SREBP2	sterol responsive element binding protein 2
TBS-T	Tris-buffered saline containing 0.05% Tween 20

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INTRODUCTION

Niemann-Pick C1-like 1 (NPC1L1) is expressed in the proximal region of the small intestine and is responsible for intestinal cholesterol absorption. The function of NPC1L1 has been demonstrated in both knockout mice (1–3) and functional analyses in *in vitro* experimental systems (4–7). NPC1L1 is believed to be a pharmacological target of ezetimibe, a novel cholesterol-lowering drug used to treat hypercholesterolemia (1,8). In addition, a recent report using liver-specific NPC1L1 transgenic mice strongly suggests the involvement of human NPC1L1 in cholesterol reuptake from bile (9).

Because NPC1L1 contributes to cholesterol (re-)absorption, cholesterol-dependent regulation of its expression is conceivable. Indeed, a high cholesterol diet reduces the intestinal expression of mouse NPC1L1 (3), and the expression of human NPC1L1 is suggested to be transcriptionally regulated by the sterol-responsive element binding protein 2 (SREBP2), which plays a key role in cholesterol-dependent downregulation, in enterocytes (10) and hepatocytes (11). Moreover, the hepatocyte nuclear factor 4α (HNF4α/NR2A1), which is a crucial modulator of lipid and glucose metabolism, has been revealed to enhance the SREBP2-dependent expression of human NPC1L1 (11).

Along with SREBP2 and HNF4α, the involvement of a number of other factors in the transcriptional regulation of NPC1L1 has been reported. Treatments with agonists of nuclear receptors, such as the peroxisome proliferator-activated receptor δ (PPARδ/NR1C2) (12,13), the liver X receptors (NR1H2 and 1H3) (14), the retinoid X receptors (RXRs/NR2Bs) (15), and bile acids (16), reduce the expression levels of NPC1L1 mRNA, although the molecular mechanisms still remain to be clarified. PPARα/NR1C1, which is also a member of the nuclear receptor superfamily and is highly expressed in the liver and intestine as a key modulator of lipid homeostasis (17), is also suggested to contribute to the regulation of mouse NPC1L1; a recent report revealed that administration of a PPARα ligand, fenofibrate, represses intestinal cholesterol absorption and the expression levels of mouse NPC1L1, and fenofibrate-sensitive regulation does not occur in PPARα-deficient mice (18). In contrast, the constitutive expression of intestinal NPC1L1 is not affected by the knockout of PPARα gene (18).

PPARα recognizes fatty acids as a physiological ligand (19), and clinical studies have shown that genetic variation in this receptor is involved in the risk and progression of diabetes (20,21). Together with the profound relationship between fatty acids and cholesterol in lipid homeostasis and the increased expression of NPC1L1 in diabetic patients (22,23), we hypothesized and characterized in the present study that PPARα and its major cofactor, PPARγ coactivator 1α (PGC1α), contribute to the transcriptional regulation of human NPC1L1.

MATERIALS AND METHODS

Materials

Restriction enzymes and KOD-plus were purchased from Takara Bio Inc. (Shiga, Japan). A Dual-Luciferase Reporter Assay System, pGEM-T Easy Vector System, pGL3-Basic and pRL-SV40 vectors were purchased from Promega KK (Tokyo, Japan). The pcDNA3.1(+) vector, phenol red-free Dulbecco's Modified Eagle Medium (DMEM) and penicillin-streptomycin were purchased from Invitrogen (Carlsbad, CA). Charcoal-absorbed fetal bovine serum was purchased from HyClone (Logan, UT). Quick Change Mutagenesis Kits were purchased from Stratagene (La Jolla, CA). FuGENE6 was purchased from Roche Diagnostics (Tokyo, Japan). Fenofibrate was purchased from Sigma-Aldrich Japan KK (Tokyo, Japan). All other chemicals used were commercially available and of reagent grade.

Vector Construction of Reporter-Linked Human NPC1L1 Promoters

A pGEM-T Easy Vector containing a fragment of about 1.3 kbp of the promoter region of human NPC1L1 (nucleotides −1315 to +20) was obtained as described previously (11). Using this plasmid as a template, additional PCR was performed to insert the genome sequences between Kpn I and Hind III sites in a pGL3-Basic vector. The resulting plasmids were referred to as p1315/Luc containing the region from −1315 to +20 of human NPC1L1 promoter, p1110/Luc containing the region from −1110 to +20, p821/Luc containing the region from −821 to +20 and p264/Luc containing the region from −264 to +20.

Two predicted PPARα-response elements (PPREs) in p1315/Luc were mutated by site-directed mutagenesis. For the production of p1315mut/Luc, sense and antisense primers used were 5′-ttaccaaatgCCTAGCGCCTAGCtggcc catcc-3′ and 5′-ggatgggccaGCTAGGCGCTAGGcatttggtaa-3′, respectively, for mutations in the sequence between −846 and −834, and, 5′-tatctgcagcGCTAGGCGCTAGG tgggggccca-3′ and 5′-tgggcccccaCCTAGCGCCTAGCgctg cagata-3′, respectively, for mutations in the sequence between −879 and −867.

Construction of Expression Vectors for Nuclear Receptors

Human PPARα cDNA was obtained as a commercially available cDNA clone (GenBank accession number

CU013435, IMAGE: 100000404) and was amplified by PCR with an Nhe I site attached at the 5′-end and a Kpn I site attached at the 3′-end, and inserted into the Nhe I / Kpn I sites in a pcDNA3.1(+) vector. Mouse PGC1α cDNA (GenBank accession number NM_008904) was amplified by PCR from total RNA of mouse liver with a BamHI site attached at the 5′-end and an Xho I site attached at the 3′-end, and inserted into the BamHI / Xho I sites in a pcDNA3.1(+) vector. Expression vectors for human HNF4α (24), human RXRα (25) and a nuclear form of human SREBP2 (11) were obtained as described previously.

Luciferase Assay

HepG2 cells were plated on day 0 at a density of 1.5×10^5 cells/well on 24-well plates and grown in phenol red-free DMEM with 10% charcoal-absorbed fetal bovine serum and 1% penicillin–streptomycin. On day 1, cells were transfected with 500 ng/well of pGL3-Basic vectors, with or without the human NPC1L1 promoter, using FuGENE6 at a DNA/lipid ratio of 1:3. In some experiments, cotransfections were performed with PPARα (100 ng/well), RXRα (100 ng/well), HNF4α (250 ng/well), SREBP2 (100 ng/well) and PGC1α (250 ng/well) in a pcDNA3.1(+) vector. All wells were also cotransfected with 50 ng/well of pRL-SV40 vector to correct the transfection efficiency. At 48 h after transfection, luciferase activity was quantified using a Luminescencer MCA (Atto, Tokyo, Japan) and the Dual-Luciferase Reporter Assay System according to the manufacturer's protocol. All results were confirmed by additional experiments.

Electrophoretic Mobility Shift Assay

Electrophoretic mobility shift assays (EMSAs) were performed with *in vitro* translated proteins and a DIG Gel Shift Kit (Roche Diagnostics) according to the manufacturer's protocol. PPARα and RXRα proteins were synthesized using TNT Quick Coupled Transcription/Translation Systems (Promega KK). The strand sequences for human NPC1L1 PPRE (between −846 and −834) were 5′-ttaccaaatgGGGGTAAAGGTCAtggcccatcc-3′ and 5′-ggatgggccaTGACCTTTACCCCcatttggtaa-3′. Mutated fragments were the same as the primers used for the introduction of mutations in the promoter constructs. Double-stranded oligonucleotides were end-labeled with digoxigenin-11-ddUTP and incubated with PPARα and/or RXRα proteins at 20°C for 2.5 h in the presence or absence of 200-fold excess amounts of unlabeled fragments of the wild-type or mutated PPRE. The mixture was applied to a native polyacrylamide gel and electrophoretically separated. Then, the oligonucleotide-protein complexes were transferred onto a positively charged nylon membrane by electroblotting, followed by detection with anti-digoxigenin antibody.

Transfection of Cells with siPPARα

To determine the effect of PPARα-knockdown on the expression of endogenous NPC1L1, HepG2 cells were suspended in DMEM so that 1 ml contained 100,000 cells. Twenty pmol/well RNAi targeting the human PPARα sequence (siPPARα, 5′-GCUUUGGCUUUACG GAAUATT-3′ and 5′-UAUUCCGUAAAGC CAAAGCTT-3′) or siControl, designed not to interfere with any human genes (Sigma-Aldrich Japan KK), were diluted in 200 μl/well of DMEM in 12-well plates. Then, 2 μl/well of Lipofectamine RNAiMAX (Invitrogen) was added to each well containing the diluted siRNA molecules. After incubation for 20 min at room temperature, 1 ml/well of diluted cells were added. One hundred and twenty hours after transfection, cells were harvested with RNA-Solv Reagent (Omega Bio-tek, Doraville, GA) for real-time quantitative PCR or were lysed with RIPA buffer (0.1% sodium dodecyl sulfate (SDS), 0.5% deoxycholate, and 1% Nonidet P-40, pH 7.4) for Western blot analysis.

Real-Time Quantitative PCR

The extracted RNA was reverse-transcribed with ReverTra Ace (Toyobo, Osaka, Japan). Real-time quantitative PCR was performed using 2 × SYBR Green (Stratagene) and Chromo4 (Bio-Rad, Tokyo, Japan) at 95°C for 10 min followed by 40 cycles at 95°C for 15 s, 50°C for 30 s and 72°C for 40 s. Primers for human NPC1L1 (sense and antisense primers were 5′-ggtatcactggaagcgagtc-3′ and 5′-aggtagaaggtggagtcgag-3′, respectively), human PPARα (sense and antisense primers were 5′-gagatttcgcaatccatcgg-3′ and 5′-ctacattcgatgttcaatgc-3′, respectively) and human β-actin (sense and antisense primers were 5′-ttcaacaccc cagccatgtacg-3′ and 5′-gtggtggtgaagctgtagcc-3′, respectively) were used.

Western Blot Analysis

Forty micrograms of total cell lysate protein diluted with 2 × SDS loading buffer was separated on 7% SDS-polyacrylamide gel with a 4.4% stacking gel. The molecular weight was determined by a prestained protein marker (New England BioLabs, Beverly, MA). Proteins were transferred electrophoretically onto an Immobilon-P transfer membrane (Millipore, Billerica, MA) using a blotter (Bio-Rad) at 15 V for 1 h. The membrane was blocked with Tris-buffered saline containing 0.05% Tween 20 (TBS-T) and 3% bovine serum albumin (BSA) for 1 h at room temperature. After washing with TBS-T, the membrane

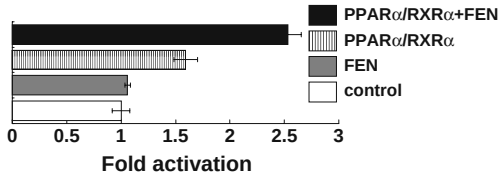


Fig. 1 Activation of the reporter-linked human NPC1L1 promoter by PPARα and fenofibrate. HepG2 cells were transfected with a luciferase-linked human NPC1L1 promoter construct (p1315/Luc) with or without expression vectors for PPARα and RXRα. Twenty-four hours later, cells were incubated for an additional 24 h in the presence or absence of 250 μM fenofibrate (FEN). The fold activation values were calculated by dividing the luciferase activity in each experiment by that of control cells. Values are expressed as the mean ± S.E. ($n = 3$).

was incubated for 1 h at room temperature in TBS-T containing 0.1% BSA with 200-fold diluted rabbit anti-NPC1L1 antibody (Cayman Chemical, Ann Arbor, MI) or 1000-fold diluted rabbit anti-α-tubulin antibody (Abcam, Cambridge, MA). For detection, the membrane was allowed to bind to 5000-fold diluted horseradish peroxidase-labeled anti-rabbit IgG antibody (Amersham Biosciences, Piscataway, NJ) in TBS-T containing 0.1% BSA for 1 h at room temperature. The enzyme activity was assessed using an ECL Plus Western blotting Detection System (Amersham Biosciences) with a luminescent image analyzer (Bio-Rad).

RESULTS

PPARα Upregulates the Transcriptional Activity of Human NPC1L1

To elucidate the PPARα-mediated regulation of human NPC1L1, we performed reporter gene assays with approximately 1.3 kbp upstream of the human NPC1L1 gene. A luciferase-linked human NPC1L1 promoter construct (p1315/Luc) was transiently transfected into HepG2 cells with expression vectors for PPARα and its heterodimer partner, RXRα, and cells were treated with a PPARα ligand fenofibrate. As shown in Fig. 1, luciferase activity was increased by cotransfection with PPARα/RXRα and further activated by fenofibrate treatment. These results indicate that PPARα signaling positively regulates transcription of the human NPC1L1 gene.

Response to PPARα Diminishes with Deletion of the Region Between −1110 and −821 in the Human NPC1L1 Promoter

To determine the region responsible for PPARα-mediated regulation, we constructed reporter plasmids with different lengths of the 5′-flanking regions of the human NPC1L1 gene (p1315/Luc, p1110/Luc, p821/Luc and p264/Luc). These constructs were transiently transfected into HepG2 cells, and the response to PPARα signaling was determined. The response to PPARα/RXRα and fenofibrate of the p1315/Luc and p1110/Luc constructs were almost the same, whereas the p821/Luc and p264/Luc constructs exhibited no response (Fig. 2). These results suggest that the regulatory elements of PPARα are located between −1110 and −821 upstream of the human NPC1L1 gene.

Mutations in a Putative PPRE Decrease PPARα-Sensitive Transcriptional Activity

Direct repeat-1 (DR-1)-like elements, which are reported to be a putative PPRE (26), were searched for in the region between −1110 and −821 upstream of the human NPC1L1 gene, and two candidate sequences were found. To determine whether these elements are functional PPREs, mutations were introduced in the DR-1 motifs by site-directed mutagenesis. Luciferase assays revealed that mutations in a putative PPRE (−846/−834) resulted in a reduction in PPARα-dependent response (Fig. 3), whereas mutations in the other putative PPRE (PPRE*, −879/−867) had little effect on the PPARα-mediated activation (Fig. 3). These observations indicate that a DR-1 motif (−846/−834) plays a crucial role in the PPARα-dependent transcriptional regulation of human NPC1L1.

PPARα/RXRα Directly Binds to the PPRE in the Human NPC1L1 Promoter

EMSAs were performed to detect a direct binding of PPARα/RXRα to the putative PPRE (−846/−834), which was found in the reporter gene analysis. As shown in Fig. 4,

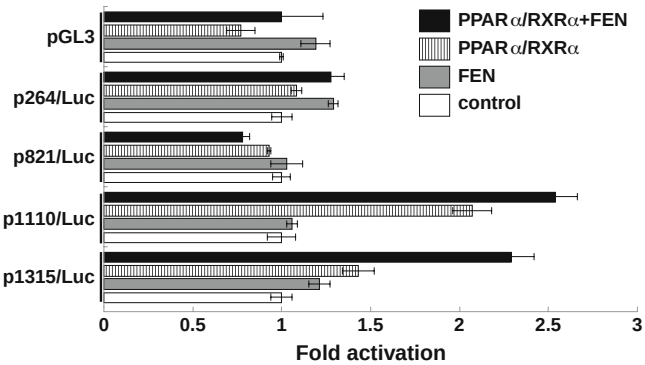
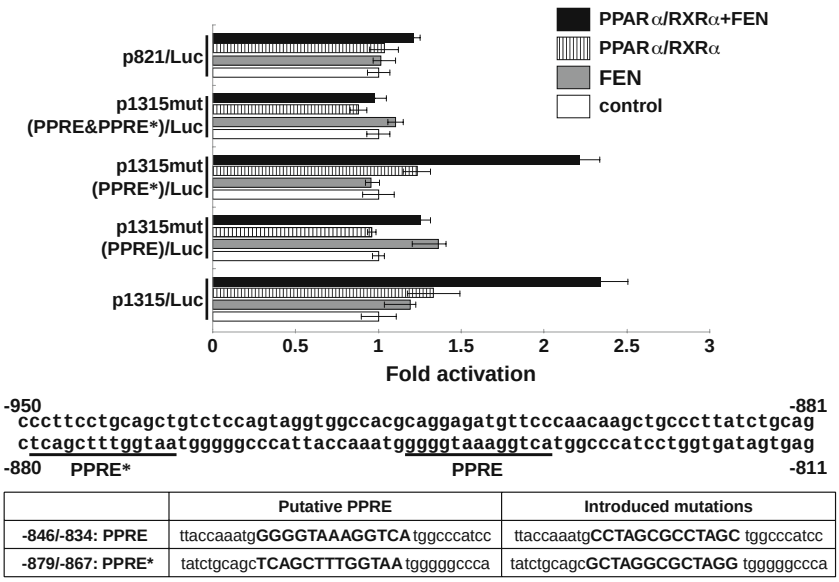


Fig. 2 Luciferase activity of deletion mutants of human NPC1L1 promoter. HepG2 cells were transfected with deletion mutants of human NPC1L1 promoter constructs (p1315/Luc, p1110/Luc, p821/Luc and p264/Luc) with or without expression vectors for PPARα and RXRα. Twenty-four hours later, cells were incubated for an additional 24 h in the presence or absence of 250 μM fenofibrate (FEN). The fold activation values were calculated by dividing the luciferase activity of each promoter by that of control cells. Values are expressed as the mean ± S.E. ($n = 3$).



	Putative PPRE	Introduced mutations
-846/-834: PPRE	ttaccaaatg**GGGGTAAAGGTCA** tggcccatcc	ttaccaaatg**CCTAGCGCCTAGC** tggcccatcc
-879/-867: PPRE*	tatctgcagc**TCAGCTTTGGTAA** tgggggccca	tatctgcagc**GCTAGGCGCTAGG** tgggggccca

Fig. 3 Effect of mutations in putative PPREs on the reporter gene activity. Mutations were introduced in the luciferase-linked human NPC1L1 promoter construct (p1315/Luc). For mutations between −846 and −834, GGGGTAAAGGTCA was replaced by CCTAGCGCCTAGC, and for mutations between −879 and −867, TCAGCTTTGGTAA was replaced by GCTAGGCGCTAGG. HepG2 cells were transfected with each human NPC1L1 promoter construct with or without expression vectors for PPARα and RXRα. Twenty-four hours later, cells were incubated for an additional 24 h in the presence or absence of 250 μM fenofibrate (FEN). The fold activation values were calculated by dividing the luciferase activity of each promoter by that of control cells. Values are expressed as the mean ± S.E. ($n = 3$).

a band-shift of the PPRE-containing DNA probe was observed when incubated with both of PPARα and RXRα proteins; however, no shift was detected with PPARα or RXRα alone. In addition, the band-shift disappeared in the presence of excess unlabeled fragments, whereas mutated fragments had no effect on the binding, and PPARα/RXRα did not form a complex with the mutated probe (Fig. 4). Together with the results of luciferase assays, the presence of a functional element for PPARα-mediated transactivation was identified in the region between −846 and −834 upstream of the human NPC1L1 gene.

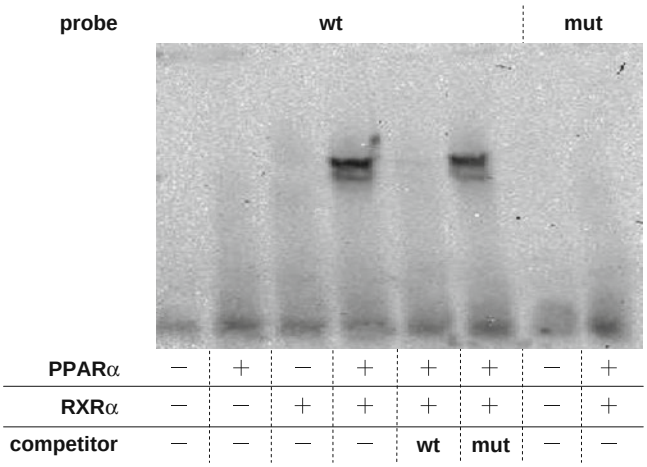


Fig. 4 Binding of PPARα/RXRα on a DR-1 motif in human NPC1L1 promoter. EMSAs were performed for a DR-1-like element in the human NPC1L1 promoter. A 5′-flanking region of the human NPC1L1 gene containing a putative PPRE (−846/−834) of the wild-type (wt) or mutated sequences (mut) was labeled with digoxigenin-11-ddUTP and used as a probe. Mutations were introduced in the region between −846 and −834; GGGGTAAAGGTCA was replaced by CCTAGCGCCTAGC. The labeled DNA fragments were incubated with PPARα and/or RXRα proteins with or without a competitor. As a competitive inhibitor, 200-fold excess amounts of unlabeled fragments of the wild-type (wt) or mutated (mut) sequences were used.

PPARα-Specific Knockdown Decreases the Expression of NPC1L1 in HepG2 Cells

To reveal the importance of PPARα in the endogenous expression of human NPC1L1, PPARα-specific siRNA was introduced into HepG2 cells. Compared with control cells, the expression of PPARα mRNA was reduced to 5.9±0.6% in siPPARα-transfected cells (Fig. 5A). Under this circumstance, a significant decrease was observed in NPC1L1 mRNA (49±4%)(Fig. 5A) and NPC1L1 protein (37±5%) (Fig. 5B), suggesting that PPARα contributes to the baseline expression of human NPC1L1.

PGC1α Stimulates SREBP2/HNF4α- and PPARα/RXRα-Mediated Activation of the Human NPC1L1 Promoter

Because PGC1α is a coactivator of transcriptional factors, including HNF4α and PPARα (27), the effects of PGC1α on the transcriptional regulation of human NPC1L1 via

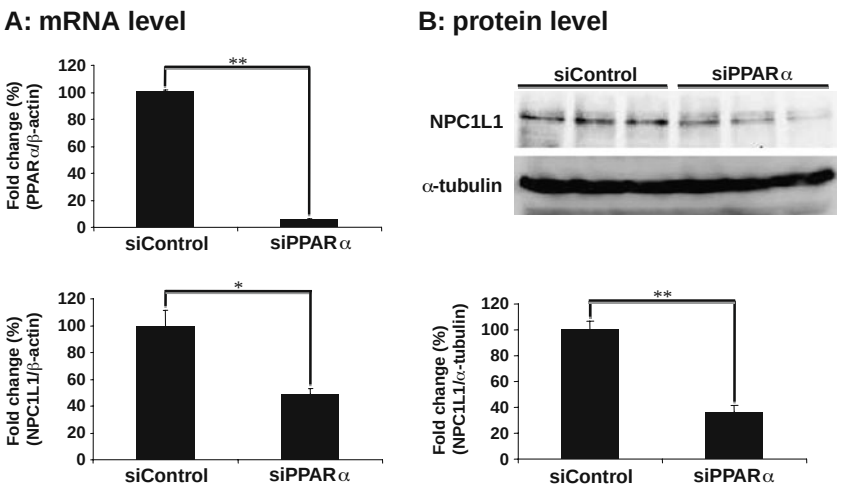


Fig. 5 Effect of siPPARα on the expression of NPC1L1 in HepG2 cells. The effect of siRNA on the endogenous expression of PPARα mRNA, NPC1L1 mRNA (**A**) and NPC1L1 protein (**B**) was determined. HepG2 cells were transfected with PPARα-specific siRNA (siPPARα) or control siRNA (siControl), and, 120 h later, RNA and protein were extracted for quantitative PCR and Western blot analyses, respectively. The fold changes were calculated as the relative expression levels compared with that of control cells. Values are expressed as the mean ± S.E. ($n=3$). *Significantly different from control cells by Student's t test ($p<0.05$). **Significantly different from control cells by Student's t test ($p<0.01$).

SREBP2/HNF4α and PPARα/RXRα pathways were examined. Results of a luciferase assay showed that cotransfection of PGC1α further increased the transactivation by both SREBP2/HNF4α (Fig. 6A) and PPARα/RXRα (Fig. 6B), suggesting the involvement of PGC1α in the transcriptional regulation of human NPC1L1.

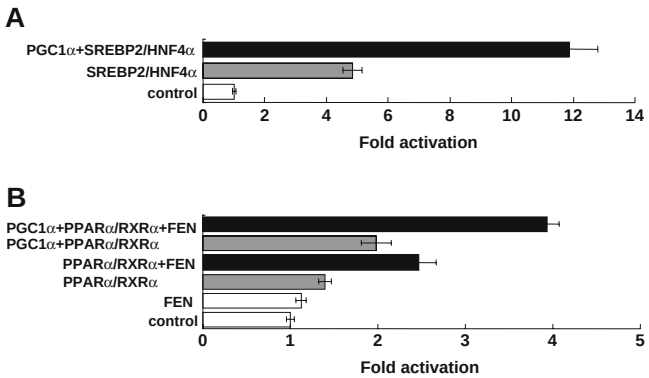


Fig. 6 Stimulatory effect of PGC1α on SREBP2/HNF4α- and PPARα/RXRα-mediated activation of human NPC1L1 transcription. (**A**) HepG2 cells were transfected with a luciferase-linked human NPC1L1 promoter construct (p1315/Luc) with indicated expression vectors for SREBP2, HNF4α and PGC1α for 48 h. The fold activation values were calculated by dividing the luciferase activity in each experiment by that of control cells. Values are expressed as the mean ± S.E. ($n=3$). (**B**) HepG2 cells were transfected with a luciferase-linked human NPC1L1 promoter construct (p1315/Luc) with indicated expression vectors for PPARα, RXRα and PGC1α. Twenty-four hours later, cells were incubated for an additional 24 h in the presence or absence of 250 μM fenofibrate (FEN). The fold activation values were calculated by dividing the luciferase activity in each experiment by that of control cells. Values are expressed as the mean ± S.E. ($n=3$).

DISCUSSION

In the present study, the transcriptional regulation of human NPC1L1 via a PPARα-mediated pathway was demonstrated. Results of reporter gene assays (Figs. 1, 2 and 3) and EMSAs (Fig. 4) revealed a DR-1-like element (−846/−834) as a functional PPRE, which differs from the previously reported HNF4α-binding DR-1 motifs (−209/−197 and −52/−40) (11).

Using a genetic knockout model, Valasek *et al.* clearly showed that PPARα acts as a negative regulator of NPC1L1 expression in mice (18); treatments with fenofibrate resulted in the reduced expression of NPC1L1 in wild-type mice but not in PPARα-knockout mice. This result is not consistent with our findings that PPARα is a transcriptional stimulator of human NPC1L1. In addition, PPARα-deficiency scarcely affected the baseline expression levels of mouse NPC1L1 (18), which is also contradictory to our results that show PPARα-knockdown causes a significant reduction in human NPC1L1 expression (Fig. 5). From these discrepancies, we assume that species differences in PPARα-mediated regulation of NPC1L1 expression exist, which has been suggested for a number of other target genes of PPARα (28). Indeed, from sequence analyses, the corresponding PPRE was not identified in the mouse NPC1L1 promoter (~4 kbp), whereas the proximal promoter region around the binding sites of SREBP2 and HNF4α are highly conserved in mice and humans. Furthermore, ezetimibe-sensitive cholesterol absorption was demonstrated to be significantly decreased by treatment with fenofibrate in mice (18), while in humans the cholesterol-lowering effects of ezetimibe tend to be rela-

tively stronger when co-administered with fenofibrate (29,30), which is consistent with the results of the present study for the transcriptional regulation of human NPC1L1. Because the potential cholesterol-lowering effect of fenofibrate and the intricate feedback regulation of lipid homeostasis cause difficulties in the interpretation of observations, further analyses are needed to obtain a definite conclusion.

For the PPARα-mediated regulation of human NPC1L1 expression, experiments using human-derived Caco-2 cells have been reported to show that the endogenous expression of NPC1L1 mRNA is not affected by treatment with PPARα agonists (12,14). In the present study using HepG2 cells, we also found that fenofibrate treatment without exogenous expression of PPARα failed to stimulate the transcriptional activities of the human NPC1L1 promoter (Fig. 1). Together with our observations in overexpression (Fig. 1) and knockdown (Fig. 5) experiments, the insubstantial response to fenofibrate in Caco-2 and HepG2 cells may be accounted for by the assumption that enough endogenous PPARα ligands are present for NPC1L1 expression.

In addition, the relationship between the expression of NPC1L1 and fatty acid metabolism should be discussed. It was recently demonstrated that overexpression of the intestinal-fatty acid binding protein (I-FABP/FABP2) resulted in a dramatic decrease in the endogenous expression of NPC1L1 protein in human intestinal epithelial cells (31). Moreover, in genetic deficient mice of CD36, which mediates the intestinal absorption of fatty acids (32), the intestinal expression of NPC1L1 protein was drastically increased (33). Considering that HNF4α and PPARα, both of which are involved in the transcriptional regulation of NPC1L1, recognize fatty acids as one of their physiological ligands, fatty acids are likely to be profoundly involved in and tightly controlling the cellular expression of NPC1L1. The presence of these regulations may be related to the fact that cholesterol, a substrate of NPC1L1, is esterified with fatty acids to be converted to cholesterol ester for storage and transfer.

Clinically, it is well known that dyslipidemia and diabetes are closely related syndromes. The elevated expression of NPC1L1 mRNA in diabetic patients (22,23) may result in an increase in serum cholesterol levels, although the precise regulatory mechanism for NPC1L1 induction remains unclear. In the present study, it was demonstrated that PGC1α, which has increased gastrointestinal expression under diabetic conditions (27), has a stimulatory effect on the human NPC1L1 promoter via SREBP2/HNF4α- and PPARα/RXRα-mediated transcription (Fig. 6). Because HNF4α and PPARα are also involved in the pathogenesis of diabetes (20,21,27), it is possible that these factors play important roles in the regulation of NPC1L1 expression under (patho)physiological conditions.

CONCLUSION

In the present study, we found that PPARα positively regulates the expression of human NPC1L1 by directly binding to a PPRE at −846/−834 upstream of the gene. Additionally, PGC1α is suggested to have stimulatory effects on the SREBP2/HNF4α- and PPARα/RXRα-mediated transactivation of the human NPC1L1 promoter. Taken together with the involvement of HNF4α, PPARα and PGC1α in the pathogenesis of diabetes (20,21,27), these findings may provide new insights into the close relationship of glucose, fatty acids and cholesterol homeostasis.

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