Cloning and functional analysis of human acyl coenzyme A: Cholesterol acyltransferase1 gene P1 promoter

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Received May 29, 2015; Accepted April 13, 2016

DOI: 10.3892/mmr.2016.5295

Abstract. Acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1) catalyzes the conversion of free cholesterol (FC) to cholesterol ester. The human ACAT1 gene P1 promoter has been cloned. However, the activity and specificity of the ACAT1 gene P1 promoter in diverse cell types remains unclear. The P1 promoter fragment was digested with *Kpn*I/*Xho*I from a P1 promoter cloning vector, and was subcloned into the multiple cloning site of the Firefly luciferase vector pGL3-Enhancer to obtain the construct P1E-1. According to the analysis of biological information, the P1E-1 plasmid was used to generate deletions of the ACAT1 gene P1 promoter with varying 5' ends and an identical 3' end at +65 by polymerase chain reaction (PCR). All the 5'-deletion constructs of the P1 promoter were identified by PCR, restriction enzyme digestion mapping and DNA sequencing. The transcriptional activity of each construct was detected after transient transfection into THP-1, HepG2, HEK293 and Hela cells using DEAE-dextran and Lipofectamine 2000 liposome transfection reagent. Results showed that the transcriptional activity of the ACAT1 gene P1 promoter and deletions of P1 promoter in THP-1 and HepG2 cells was higher than that in HEK293 and HeLa cells. Moreover, the transcriptional activity of P1E-9 was higher compared with those of other deletions in THP-1, HepG2, HEK293 and HeLa cells. These findings indicate that the transcriptional activity of the P1 promoter and the effects of deletions vary with different cell lines. Thus, the P1 promoter may drive ACAT1 gene expression with cell-type specificity. In addition, the core sequence of ACAT1 gene P1 promoter was suggested to be between -125 and +65 bp.

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Key words: acyl coenzyme A: cholesterol acyltransferase 1, promoter regions, luciferase

Introduction

Acyl-coenzyme A: cholesterol acyltransferase 1 (ACAT1) is an intracellular enzyme which catalyzes the formation of cholesteryl esters from cholesterol and long-chain fatty acyl-coenzyme A (1). In adult human tissues, ACAT1 is found in a number of different tissues and cell types, including hepatocytes and Kupffer cells, adrenal glands, neurons and macrophages, and accounts for >80% of the total ACAT enzyme activity measured *in vitro* (2-4). In addition to storing cholesterol intracellularly, ACAT1 has important physiological roles, including in hepatic lipoprotein assembly, dietary cholesterol absorption and steroidogenesis. Cholesterol and its metabolites have been implicated in the pathogenesis of multiple human diseases, including atherosclerosis, cancer, neurodegenerative diseases and diabetes. ACAT1 is important in the formation of macrophage-derived foam cells in atherosclerotic lesions (1). In addition, ACAT1 expression was indicated to serve as a potential prognostic marker in prostate cancer, specifically in differentiating indolent and aggressive forms of cancer (5). ACAT1 knockdown gene therapy was shown to decrease amyloid-β in a mouse model of Alzheimer's disease (6). In addition, ACAT1 represents a novel biomarker in adipose tissue associated with type 2 diabetes in obese individuals (7). Thus, understanding how cells maintain the homeostasis of cholesterol and its metabolites is an important area of research.

The human ACAT1 gene contains two promoters (P1 and P7), each located on a different chromosome (1 and 7) (8). Northern blot analyses have revealed the presence of four ACAT1 mRNAs (7.0-, 4.3-, 3.6- and 2.8-knt), present in almost all human tissues and cells examined (8). These messengers share the same coding sequence. The 2.8- and 3.6-knt messengers, comprising more than 70-80% of the total ACAT1 mRNAs, are produced from the P1 promoter (8). In the present study, a luciferase reporter vector containing human ACAT1 gene P1 promoter, and eight different 5'-deletion constructs of the P1 promoter was constructed to analyze the transcriptional

Table I. Sequences of the primers used in the amplification of 5'-deletion fragments of human ACAT1 gene P1 promoter.

Name	Sequence (5'-3')	Position (bp)
P1E-2		
Sense	5'-ATGATCAggtaccCAGGTTTTTTCCCCTTATC-3'	-547 to +65
Antisense	5'-GATCActcgagGTCGACTCCGGGAAGCTCTCC-3'	
P1E-3		
Sense	5'-ATGATCAggtaccTTCAAACGGTAAGGAATC-3'	-498 to +65
Antisense	5'-GATCActcgagGTCGACTCCGGGAAGCTCTCC-3'	
P1E-4		
Sense	5'-ATGATCAggtaccCTGGCTAGTTCTACG -3'	-428 to +65
Antisense	5'-GATCActcgagGTCGACTCCGGGAAGCTCTCC-3'	
P1E-5		
Sense	5'-ATGATCAggtaccGGCTTCTTCCAGTCG -3'	-363 to +65
Antisense	5'-GATCActcgagGTCGACTCCGGGAAGCTCTCC-3'	
P1E-6		
Sense	5'-ATGATCAggtaccGCTCCATGCTACCACGC-3'	-324 to +65
Antisense	5'-GATCActcgagGTCGACTCCGGGAAGCTCTCC-3'	
P1E-7		
Sense	5'-ATGATCAggtaccACATTCTACTGCTGGGGTG -3'	-256 to +65
Antisense	5'-GATCActcgagGTCGACTCCGGGAAGCTCTCC-3'	
P1E-8		
Sense	5'-ATGATCAggtaccAGCTTCCTTGGCAAGGTTGCC-3'	-188 to +65
Antisense	5'-GATCActcgagGTCGACTCCGGGAAGCTCTCC-3'	
P1E-9		
Sense	5'-ATGATCAggtaccGGGAAGGGGCGGGGAGG-3'	-125 to +65
Antisense	5'-GATCActcgagGTCGACTCCGGGAAGCTCTCC-3'	

Lower case letters represent the *KpnI*/*Xho*I linker sequences. ACAT1, Acyl-coenzyme A: Cholesterol acyltransferase 1.

function of the human ACAT1 gene P1 promoter using a luciferase reporter gene transient expression system. The present study may provide an experimental basis for investigating the regulatory mechanisms of the ACAT1 gene P1 promoter during various biological processes associated with cholesterol homeostasis, in particular the development of atherosclerosis.

Materials and methods

Materials. RPMI-1640, Dulbecco's modified Eagle's medium, and fetal calf serum were obtained from Gibco, Thermo Fisher Scientific, Inc. (Waltham, MA, USA). T4 DNA ligase, pGL3 luciferase reporter vectors, pRL-TK vector and a Dual-luciferase reporter assay system were purchased from Promega Corporation (Madison, WI, USA). Pyrobest DNA polymerase, DNA Marker DL2000, λ-EcoT14 I digest DNA Marker and all restriction enzymes were purchased from Takara Biotechnology Co., Ltd. (Dalian, China). dNTPs were obtained from MBI Fermentas, Thermo Fisher Scientific, Inc. The plasmid purification mini kit was purchased from Watson Biotechnologies, Inc. (Shanghai, China). Primers were synthesized by Invitrogen, Thermo Fisher Scientific, Inc. The Gel Extraction kit was obtained from Axygen Biosciences (Hangzhou, China). DNA sequences were analyzed using an ABI 3730 automated DNA sequence analyzer by Invitrogen, Thermo Fisher Scientific, Inc. All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Cell culture. THP-1 cells were purchased from the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China). HepG2, HEK293 and Hela cells were obtained from Institute of Biochemistry and Cell Biology (Shanghai Institutes for Biological Sciences; Chinese Academy of Sciences, Shanghai, China). HepG2, HEK293 and Hela cells were maintained in Dulbecco's modified Eagle's medium (DMEM) and THP-1 cells in RPMI-1640 medium. Culture medium was supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 U/ml penicillin (Gibco; Thermo Fisher Scientific, Inc.), and 50 μg/ml streptomycin (Gibco; Thermo Fisher Scientific, Inc.), and cells were maintained in a humidified atmosphere of 5% CO_2 and 95% air at 37˚C.

Construction of luciferase reporter vectors. The previously described cloning vector pMD19-T-P1 constructed in our laboratory (9) containing the -603/+65 region of human ACAT1 gene P1 promoter was used in this study. In the current study, the ACAT1 gene P1 promoter was isolated from the

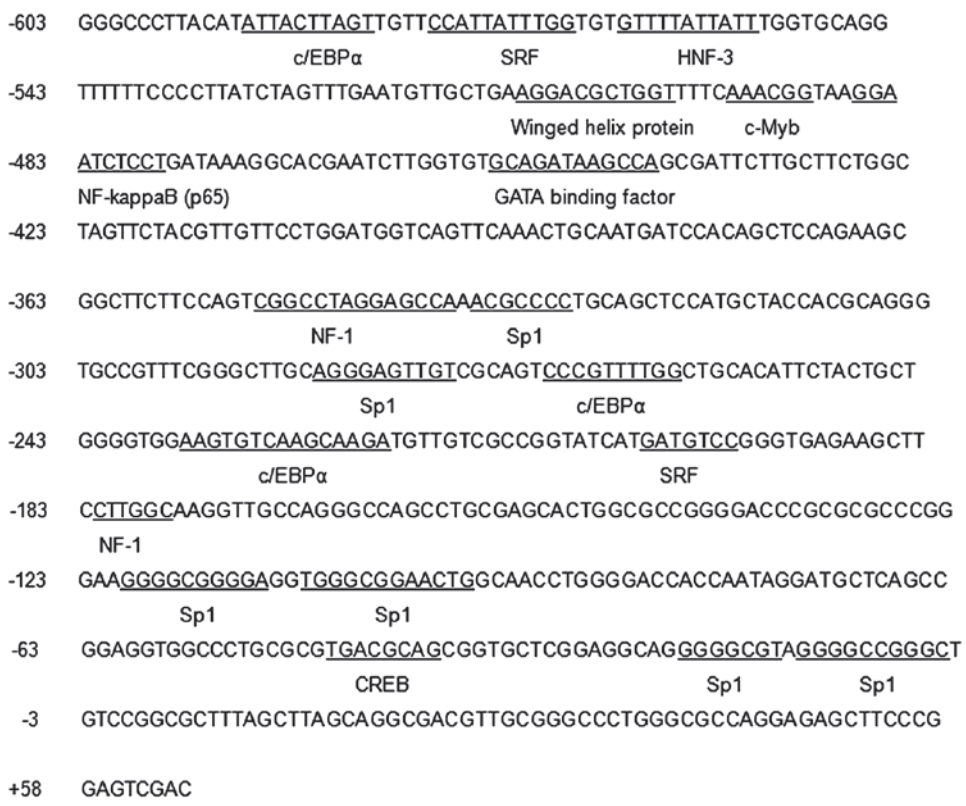

Figure 1. Biological information analysis of the human Acyl-coenzyme A: Cholesterol acyltransferase 1 gene P1 promoter. C/EBPα, CCAAT enhancer binding protein α; SRF, serum response factor; HNF-3, hepatocyte nuclear factor-3; NF-1, nuclear factor-1; DREB, cyclic AMP-response element-binding protein; NF-κB, nuclear factor-κB.

pMD19-T-P1 vector following digestion with *Kpn*I/*Xho*I, and the fragment was subcloned into the multiple cloning sites of the Firefly luciferase reporter gene vector pGL3-Enhancer to obtain P1E-1.

Transcription Element Search System (www.cbil.upenn.edu/cgi-bin/tess/tess) was used to predict transcription factor binding sites in the DNA sequence of the human ACAT1 P1 promoter. According to the analysis of biological information, the P1E-1 plasmid was used to generate deletions of the ACAT1 gene P1 promoter with varying 5' ends and an identical 3' end at +65 by polymerase chain reaction (PCR). The primer sequences, presented in Table I, were designed according to the human ACAT1 gene P1 promoter GenBank sequence (www.ncbi.nlm.nih.gov/genbank; GenBank accession no. AF143319), all included a 6-bp *Kpn*I/*Xho*I linker. All PCR reactions were performed in a total volume of 50 μl containing 1X Pyrobest buffer II, 1.5 mmol/l $MgCl_2$, 200 μmol/l deoxynucleotides, 200 nmol/l each primer, 2 units Pyrobest DNA polymerase and 0.5 μg DNA. Cycling conditions were as follows: 23 Cycles of pre-denaturation at 94˚C for 5 min, denaturation at 94˚C for 30 sec, annealing at 52˚C for 30 sec, and extension at 72˚C for 1 min (Bio-Rad T100 PCR Thermal Cycler; Bio-Rad Laboratories, Inc., Hercules, CA, USA). The PCR products were then digested with *Kpn*I and *Xho*I. The digested PCR products were analysed using agarose gel electrophoresis and then retrieved using a gel extraction kit (Axygen Biosciences). The pGL3 Enhancer plasmid was also digested by double digestion and the large fragment was retrieved. During a ligation reaction, the recycled PCR products and empty pGL3 Enhancer vector were incubated overnight with T4 DNA ligase at 4˚C, transformed into *E. coli* DH5α (Takara Biotechnology Co., Ltd.) and screened. The plasmid was extracted and identified by PCR amplification, restriction enzyme digestion mapping and DNA sequencing. The 5'-deletion constructs of the P1 promoter represent bases -547 to +65 bp (P1E-2), -498 to +65 bp (P1E-3), -428 to +65 bp (P1E-4), -363 to +65 bp (P1E-5), -324 to +65 bp (P1E-6), -256 to +65 bp (P1E-7), -188 to +65 bp (P1E-8) and -125 to +65 bp (P1E-9). These constructs were then identified by restriction enzyme digestion mapping and DNA sequencing. Sequences were determined on an ABI 3730 automated DNA sequence analyzer by Invitrogen (Thermo Fisher Scientific, Inc.). The plasmids were purified by the Wizard purification plasmid DNA purification system (Promega Corporation, Madison, WI, USA) to transfect cells.

Transient transfection. A series of luciferase reporter constructs containing the ACAT1 gene P1 promoter were transfected into THP-1 cells using the Diethylaminoethyl dextran (DEAE-dextran) method (10). Briefly, after washing twice with phosphate-buffered saline (PBS), $1x10^6$ cells were transfected with 0.5 μg ACAT1 promoter/Luc plasmids and 10 ng *Renilla* Luciferase Reporter Vector (pRL-TK) as internal control in 200 μl STBE (25 mmol/l Tris-HCl, pH 7.4; 5 mmol/l KCl, 0.7 mmol/l $CaCl_2$, 137 mmol/l NaCl, 0.6 mmol/l Na_2HPO_4 and 0.5 mmol/l $MgCl_2$) containing 60 μg DEAE-dextran. The cells were incubated for 20 min at 37˚C, washed once with RPMI-1640 without FBS, resuspended in 500 μl fresh RPMI-1640 with 10% FBS, and plated in a 24-well plate. After 48 h, the cells were harvested. The

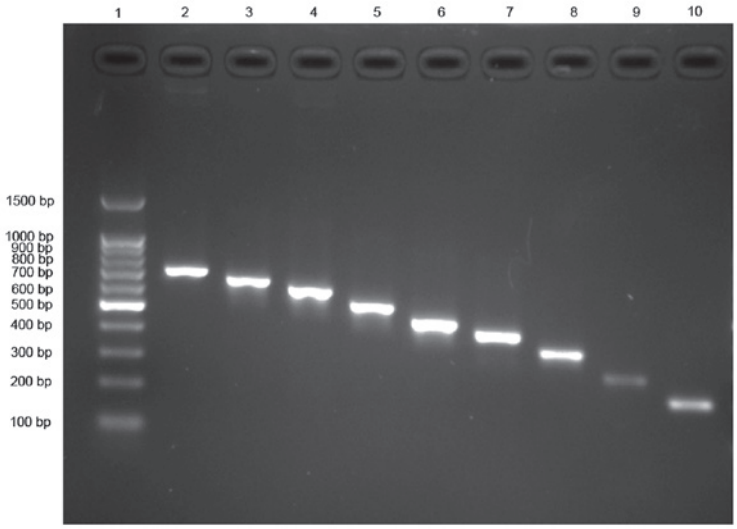

Figure 2. Fragments of the ACAT1 gene P1 promoter and its deletions amplified by PCR with the recombinant plasmid as template. Lane 1, 100 bp DNA Ladder; lane 2, PCR product of P1E-1 (with plasmid pGL3E-P1 DNA template, 685 bp); lane 3, PCR product of P1E-2 (611 bp); lane 4, PCR product of P1E-3 (562 bp); lane 5, PCR product of P1E-4 (492 bp); lane 6, PCR product of P1E-5 (472 bp); lane 7, PCR product of P1E-6 (388 bp); lane 8, PCR product of P1E-7 (320 bp); lane 9, PCR product of P1E-8 (252 bp);and lane 10, PCR product of P1E-9 (189 bp). PCR, polymerase chain reaction; ACAT1, acyl coenzyme A: Cholesterol acyltransferase 1.

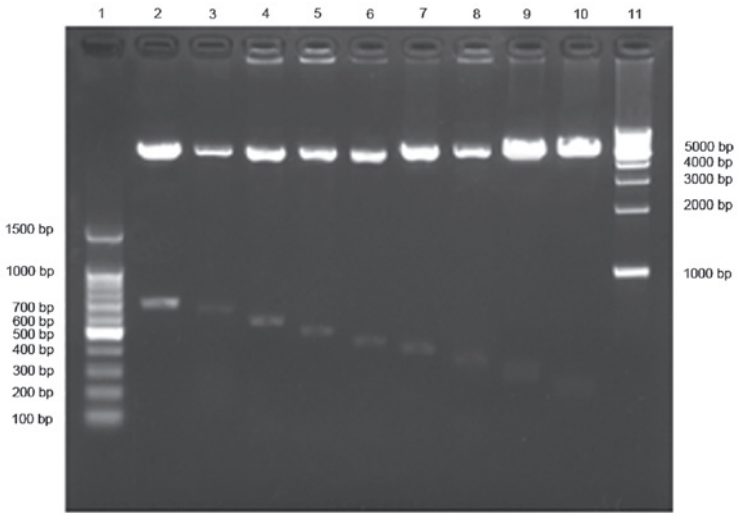

Figure 3. Identification of the recombinant plasmids of ACAT1 gene P1 promoter and its deletions by enzyme digestion. Lane 1, 100 bp DNA Ladder; lane 2, P1E-1 (pGL3E-P1, 685 bp); lane 3, P1E-2 (611 bp); lane 4, P1E-3 (562 bp); lane 5, P1E-4 (492 bp); lane 6, P1E-5 (472 bp); lane 7, P1E-6 (388 bp); lane 8, P1E-7 (320 bp); lane 9, P1E-8 (252 bp); lane 10, P1E-9 (189 bp); and lane 11, 1 kbp DNA Ladder Marker. All the recombinants were digested with *Kpn*I/*Xho*I restriction enzymes. ACAT1, acyl coenzyme A: Cholesterol acyltransferase 1.

series of luciferase reporter constructs containing the ACAT1 gene P1 promoter were separately transfected into HepG2, HEK-293 and HeLa cells using Lipofectamine 2000 liposome (Invitrogen, Thermo Fisher Scientific, Inc.) according to the manufacturer's instructions. Briefly, the day prior to transfection, cells reached 80-90% confluence. Then, the cells were transfected with the plasmid DNA and Lipofectamine 2000 at a ratio of 0.8 μg DNA:2 μl lipid per well in serum free Opti-minimal essential medium (Gibco; Thermo Fisher Scientific, Inc.) at 37˚C for 4 h. Cells were then grown for 48 h in DMEM containing FBS. All the cells were transfected with pGL3-Enhancer and pGL3-Control, which served as negative and positive controls, respectively. Each transfection reaction was performed in triplicate.

Luciferase assay. The transfected cells were washed twice with cold phosphate-buffered saline (PBS) and lysed in 100 μl passive lysis buffer (Promega Corporation, Madison, WI, USA) and centrifuged at 12,000 x g for 30 sec at 4˚C after placing at room temperature for 15 min. Then, 20 μl of the cell lysate was mixed with 100 μl Luciferase Assay Reagent II (Promega Corporation) for luciferase activity measurement in a Lumat LB9507 luminometer (EG&G Bertold, Freiburg, Germany), 100 μl Stop & Glo Reagent (Promega Corporation) was then added for luciferase activity measurement. For the luciferase activity assay, the Dual-luciferase reporter assay system (Promega Corporation) was used. Results were obtained from three different transfection experiments after normalization to the

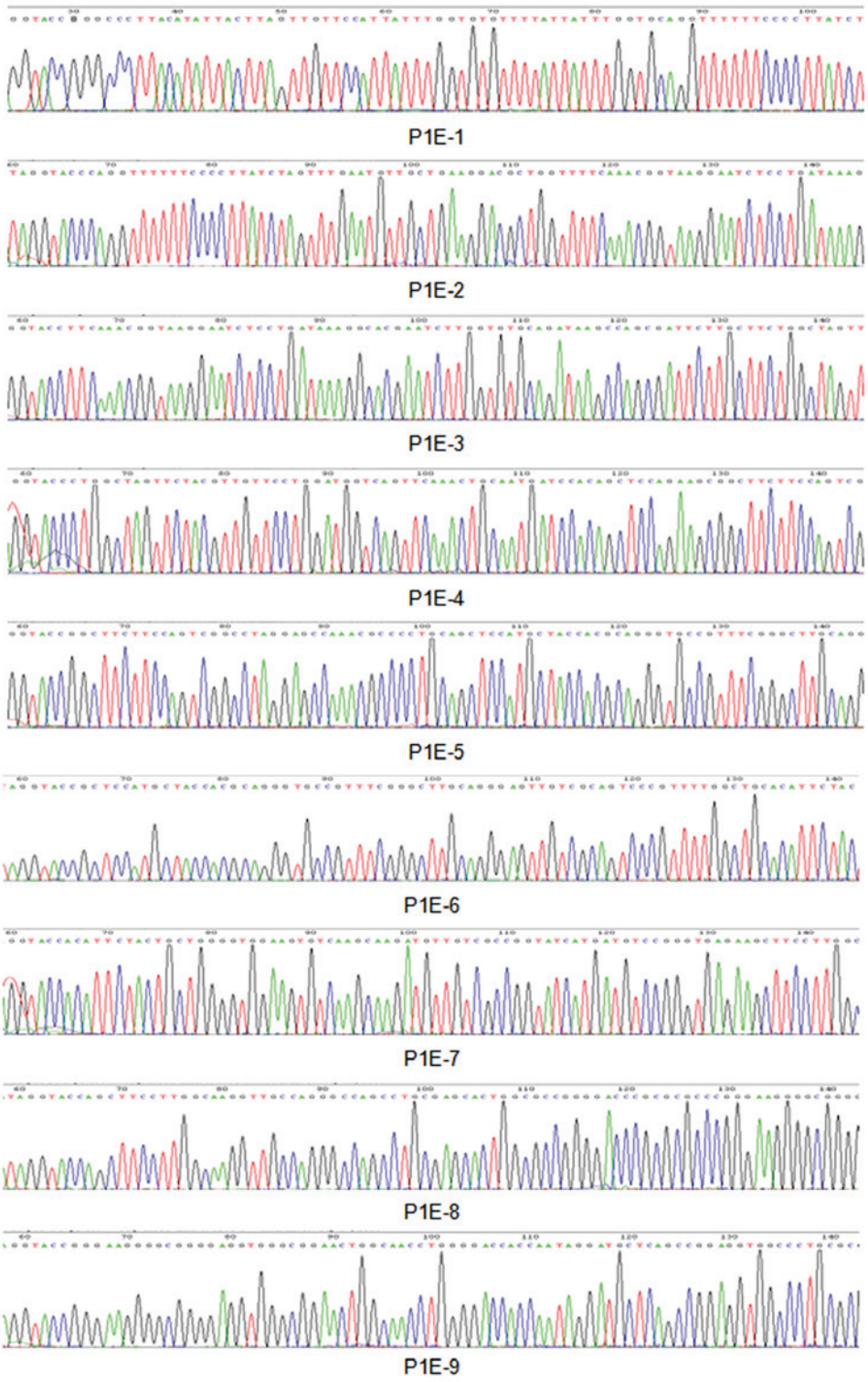

Figure 4. Partial sequencing of the acyl coenzyme A. Cholesterol acyltransferase 1 gene P1 promoter and its deletions. The DNA sequencing of P1E-1, P1E-2, P1E-3, P1E-4, P1E-5, P1E-6, P1E-7, P1E-8 and P1E-9 were consistent with GenBank database (accession AF143319).

internal control, thymidine kinase activity. Experimental variations are indicated as the mean ± standard deviation.

Statistical analysis. Results are presented are the mean ± standard deviation from at least three independent experiments performed in duplicate. Statistical analysis was performed using one-way analysis of variance and Tukey's post-hoc test with SPSS software (version 12.0; SPSS, Inc., Chicago, IL, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Biological information analysis of human ACAT1 gene P1 promoter. The ACAT1 gene P1 promoter is contiguous with the coding sequence and spans from -603 to +65 of

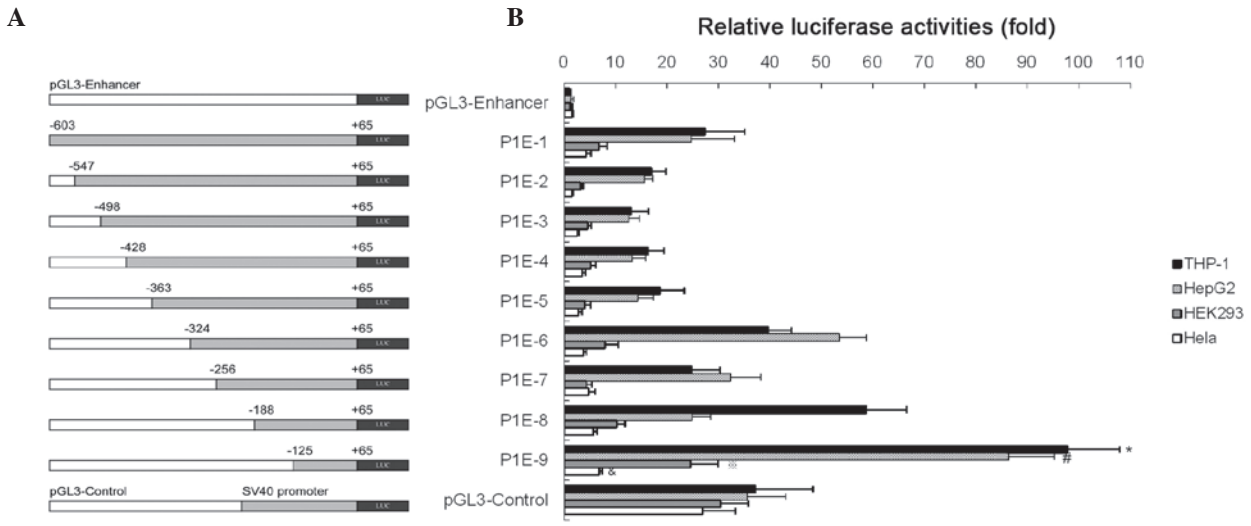

Figure 5. Effects of deletions on the transcription activities of the ACAT1 gene P1 promoter in different cell lines. (A) Schematic presentation of the ACAT1 gene P1 promoter region and its eight truncated forms. (B) Relative luciferase activities in cells transfected with each truncated reporter construct. The transfected cells were determined by luciferase after 48 h transfection. In each set of experiments, luciferase activity was normalized to pRL-TK plasmid. The bars were presented as the mean ± standard deviation of three independent transfection experiments. *P<0.05 vs. P1E-1, P1E-2, P1E-3, P1E-4, P1E-5, P1E-6, P1E-7 or P1E-8 in THP-1 cells. #P <0.05 vs. P1E-1, P1E-2, P1E-3, P1E-4, P1E-5, P1E-6, P1E-7 or P1E-8 in HepG2 cells. %P<0.05 and &P<0.05 vs. P1E-1, P1E-2, P1E-3, P1E-4, P1E-5, P1E-6, P1E-7 or P1E-8 in HEK293 and Hela cells, respectively. ACAT1, acyl coenzyme A: Cholesterol acyltransferase 1.

the ACAT1 genomic sequence (9). Using sequence analysis (Transcription Element Search System), neither a typical TATA box nor a typical CAAT box was found. However, four copies of a typical GC box (Sp1) were found in this region. In the P1 promoter sequence, other potential binding sites for various transcription factors existed, including CCAAT enhancer binding protein α (C/EBPα), serum response factor (SRF), hepatocyte nuclear factor-3 (HNF-3), winged helix protein, c-Myb, GATA binding factor, nuclear factor-1 (NF-1), cyclic AMP-response element-binding protein and nuclear factor-κB (NF-κB). Fig. 1 shows the biological information analysis of the human ACAT1 gene P1 promoter DNA sequence.

Identification of luciferase reporter vectors. The ACAT1 gene P1 promoter and a series of 5'-deletion fragments were amplified by PCR with their recombinant constructs as templates. The electrophoresis of these PCR products showed nine different fragments in 0.9% agarose gel (Fig. 2). The recombinant constructs of P1 promoter and its deletions were identified by restriction enzyme digestion mapping. When these recombinant constructs were digested with *Kpn*I and *Xho*I, two fragments were generated, 5,037 bp pGL3-Enhancer vector and 685 bp ACAT1 gene P1 promoter in length, or 611, 562, 492, 427, 388, 320, 252 and 189 bp 5'-deletion fragments in length (Fig. 3). DNA sequencing was conducted using an ABI 3730 automated DNA sequence analyzer. The sequencing results were consistent with GenBank database. Figure 4 showed the partial sequence of the human ACAT1 gene P1 promoter.

Transcriptional activity of human ACAT1 gene P1 promoter and its deletions in diverse cell lines. The recombinant constructs of the P1 promoter and its deletions, including P1E-1, P1E-2, P1E-3, P1E-4, P1E-5, P1E-6, P1E-7, P1E-8 and P1E-9 were transfected in THP-1, HepG2, HEK-293 and HeLa cells, respectively. The transcriptional activities were detected by a luciferase activity assay analysis method. pGL3-Enhancer and pGL3-Control served as negative and positive controls respectively. The results (Fig. 5) demonstrated that the activity of the ACAT1 gene P1 promoter with and without deletions in THP-1 cells was increased compared with that in HepG2, HEK293 and HeLa cells. Low activity was detected in HEK293 and HeLa cells transfected with ACAT1 gene P1 promoter and its deletions. As shown in Fig. 5, the luciferase activity of P1E-9 was greater than that of the other deletions when transfected into THP-1, HepG2, HEK293 and HeLa cells. Moreover, the activity of P1E-9 was highest when it was transfected into THP-1 cells, which was 97.7 fold higher than the pGL3-Enhancer. The maximal transcriptional activity was located within the 189-base pair region from -125 to +65, which suggested that the core sequence of ACAT1 gene P1 promoter was located in this region. Multiple GC boxes, which are likely to be Spl binding sites are present within this region (Fig. 1). The luciferase activities of P1E-1 were notably higher than those of P1E-2 in THP-1, HepG2, HEK-293 and HeLa cells, which suggested that the region from the -603 to -547 may contain positive *cis*-acting regulatory element(s). The luciferase activity of P1E-2, P1E-3, P1E-4 and P1E-5 were markedly lower than that of P1E-6, P1E-7, P1E-8 and P1E-9 in THP-1, HepG2, HEK 293 and HeLa cells. Thus, it was speculated that there were the transcription repressors in the sequence from -547 to -324. Further studies are required to investigate the activating or repressing function of these regions.

Discussion

Cholesterol is essential to the growth and viability of cells. In mammalian cells, the homeostasis of free cholesterol and

cholesteryl ester is strictly controlled. ACAT1 is a key enzyme in the regulation of the metabolic homeostasis of cholesterol and cholesteryl ester. The main mode of sterol-specific regulation of ACAT1 has been identified at the post-translational level, involving allosteric regulation by its substrate cholesterol (11). ACAT1, however, could also be regulated at the transcriptional level. Yang *et al* (12) showed that a glucocorticoid response element in the human ACAT1 P1 promoter responsible for the dexamethasone-induced elevation of ACAT1 gene expression could be functionally bound to the glucocorticoid receptor. In human THP-1 monocytic cells, the combination of interferon-γ and all-*trans* retinoic acid enhanced ACAT1 P1 promoter activity in a synergistic manner (10). Previous studies showed that insulin could upregulate ACAT1 expression and enzyme activity in human monocyte-derived macrophages at the transcriptional level by activating the P1 promoter (9). Other factors associated with atherosclerosis upregulate ACAT1 gene expression in human monocyte-macrophages, such as 1, 25-dihydroxyvitamin D (3) or 9-*cis*-retinoic acid, transforming growth factor-β1, serotonin, salusin-β and leptin (13-17). However, incretins, hydrogen sulfide, adiponectin and Ghrelin downregulated ACAT1 expression to suppress the development of atherosclerosis (18-21). However, the molecular mechanism of non-sterol-specific mediated ACAT1 regulation remains largely unknown.

To investigate the regulatory mechanisms of the ACAT1 gene in diverse cell lines during the disorder of cholesterol metabolism, a series of deletions of the P1 promoter were generated with varying 5' ends and an identical 3' end at +65 according to the analysis of biological information, and analyzed the functional regions of ACAT1 gene P1 promoter. A series of 5'-deletion luciferase reporter gene constructs of the P1 promoter were successfully constructed. Then the transcriptional activities of P1 promoter and its deletions, P1E-1, P1E-2, P1E-3, P1E-4, P1E-5, P1E-6, P1E-7, P1E-8 and P1E-9, were detected after transient transfection into THP-1, HepG2, HEK293 and Hela cells, respectively. The results showed that the activities of the ACAT1 gene P1 promoter and its deletions in THP-1 cells were higher than that in HepG2, HEK293 and HeLa cells. There were low activities observed when each reporter vector was transfected into HEK293 and HeLa cells respectively. It was hypothesized that different deletions of the ACAT1 gene P1 promoter have different effects on transcription activities, and that the activity varies with cell type. Smith *et al* (22) demonstrated quantitatively that ACAT1 is the predominant transcript isoform in human peripheral blood mononuclear cells and liver, which is consistent with the present results. Thus, the ACAT1 gene P1 promoter may drive gene expression with cell type specificity.

In this study, the P1 promoter characterization of the ACAT1 gene was conducted and THP-1, HepG2, HEK 293 and HeLa cell lines were selected for deletion analysis. Results from the deletion analysis of ACAT1 gene P1 promoter indicated that there were several potential regions containing positive or negative *cis*-acting regulatory element(s). The results suggested that the region from -603 to -547 may contain positive *cis*-acting regulatory element(s). Bioinformatics analysis of the region from -603 to -547 found three cis-acting regulatory elements: C/EBPα, SNF and HNF-3 binding sites. The results of deletion analysis suggested that there were transcription repressors in the region from -547 to -324. Bioinformatics analysis of the region from -547 to -324 found several *cis*-acting regulatory elements, including winged helix protein, c-Myb, NF-κB, GATA binding factor, NF-1, Sp1 binding sites. The activity of P1E-9 was notably higher than those of the other deletions in THP-1, HepG2, HEK293 and HeLa cells. Moreover, the activity of P1E-9 was highest in THP-1 cells, which was 97.7 multiple of pGL3-Enhancer. Thus, it was presumed that the core sequence of the ACAT1 gene P1 promoter was located in the region from -125 to +65. There was neither a typical TATA box nor a typical CAAT box, however, four copies of a typical GC box (Sp1) were found in this region, which was important for the transcriptional activity of the ACAT1 gene P1 promoter. The results suggested that the activities of the potential positive or negative elements of the P1 promotor in different cell lines determined the differences in ACAT1 gene expression. Further studies on the effects of site-directed mutagenesis are required for these potential transcription factor binding sites of the ACAT1 gene P1 promoter in different cell lines.

In conclusion, luciferase reporter vectors containing human ACAT1 gene P1 promoter with and without deletions were successfully constructed, and the transcriptional activities in diverse cell lines and the transcriptional activities of different regions of P1 promoter were determined. Deletion analysis revealed the core sequence of the ACAT1 gene P1 promoter and potential positive or negative *cis*-acting regulatory element(s). These finding may explain why the ACAT1 gene expression varied between cell types specificity and provided an experimental basis for investigating the regulatory mechanisms of the ACAT1 gene P1 promoter in various biological processes associated with cholesterol homeostasis, particularly the development of atherosclerosis. Further investigation is required to determine the association between cis-acting elements and the interacting trans-acting factors within the ACAT1 gene P1 promoter regulatory region. The results may contribute to the development of novel treatment strategies and/or the determination of additional therapeutic targets for the prevention and treatment of diseases associated with cholesterol metabolism imbalance.

Acknowledgements

This study was supported by the National Natural Scientific Foundation of China (grant no. 30471921 to Dr Bei Cheng and grant no. 30700767 to Dr Wei Zhai), and the Natural Scientific Foundation of Hubei province of China (grant nos. 2013CFB073 and 2013CFB088).

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