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Conditional Ablation of Mediator Subunit MED1 (*MED1/PPARBP*) Gene in Mouse Liver Attenuates Glucocorticoid Receptor Agonist Dexamethasone-Induced Hepatic Steatosis

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Glucocorticoid receptor (GR) agonist dexamethasone (Dex) induces hepatic steatosis and enhances constitutive androstane receptor (CAR) expression in the liver. CAR is known to worsen hepatic injury in nonalcoholic hepatic steatosis. Because transcription coactivator *MED1/PPARBP* gene is required for GR- and CAR-mediated transcriptional activation, we hypothesized that disruption of *MED1/PPARBP* gene in liver cells would result in the attenuation of Dex-induced hepatic steatosis. Here we show that liver-specific disruption of MED1 gene ($MED1^{\Delta Liv}$) improves Dex-induced steatotic phenotype in the liver. In wild-type mice Dex induced severe hepatic steatosis and caused reduction in medium- and short-chain acyl-CoA dehydrogenases that are responsible for mitochondrial β-oxidation. In contrast, Dex did not induce hepatic steatosis in mice conditionally null for hepatic MED1, as it failed to inhibit fatty acid oxidation enzymes in the liver. $MED1^{\Delta Liv}$ livers had lower levels of GR-regulated CAR mRNA compared to wild-type mouse livers. Microarray gene expression profiling showed that absence of MED1 affects the expression of the GR-regulated genes responsible for energy metabolism in the liver. These results establish that absence of MED1 in the liver diminishes Dex-induced hepatic steatosis by altering the GR- and CAR-dependent gene functions.

Key words: Mediator complex subunit 1 (MED1); PPARBP; Hepatic steatosis; Dexamethasone; Glucocorticoid receptor; Constitutive androstane receptor

INTRODUCITON

Nuclear receptors regulate a diverse array of biological processes, including development, differentiation, and neoplasia, as well as energy and xenobiotic metabolism (29). These receptors bind, as homo- or heterodimers, to specific response elements in target gene promoters to regulate gene expression (29). The binding of ligands to nuclear receptors initially influences the recruitment of coactivator complexes, such as members of p160 family, which exhibit histone acetyltransferase activity to facilitate chromatin remodeling (19). Subsequent docking of other cofactors, or preformed multisubunit protein complexes, including mediator complex, facilitates interaction of liganded receptors with RNA polymerase II and the general basal transcription machinery (14).

Mediator subunit 1 (MED1), which was originally identified as peroxisome proliferator-activated receptor (PPAR)-binding protein (PBP/PPARBP) (37), and

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subsequently identified as thyroid hormone receptor-associated protein 220 (TRAP220) (35), and vitamin D receptor interacting protein 205 (DRIP205) in TRAP and DRIP transcriptional complexes (28), appears to be a pivotal cofactor for the maintenance of mediator complex integrity (14). Gene knockout studies in the mouse have established that disruption of *MED1/PPARBP* gene is embryonic lethal, implying that MED1 may be widely involved in the functioning of many transcription factors (6,38). To study the function of *MED1/PPARBP* gene in adult tissues, we generated mice for conditional gene disruption using the Cre-loxP strategy (8). Evidence obtained to date from conditional disruption of this gene in the liver has established that MED1 is essential for PPARα and constitutive androstane receptor (CAR)-regulated gene expression in the liver (8,9).

Because CAR expression is glucorcorticoid receptor (GR) dependent and that a functional glucocorticoid response element (GRE) is present in the CAR gene promoter (24), it is envisaged that MED1 deficiency in vivo may affect the transcription of GR target genes in response to GR ligand dexamethasone (Dex) (24). To examine this aspect, we decided to study the role of MED1 in Dex-induced hepatic steatosis (16,20). Dex, a synthetic compound of glucocorticoid class of steroid hormones, used widely as an anti-inflammatory and immunosuppressant agent, is known to enhance the expression of CAR in the liver (24). In humans, as well as in rodents, treatment with Dex causes hepatic steatosis, by mechanisms that are not well understood (16). Dex-activated GR is transported to the nucleus where it binds as homodimer to GRE sequences in target gene promoters (16). Dex increases the levels of CAR, pregnane X receptor (PXR) and retinoid X receptor (RXR) mRNAs and proteins, to potentiate xenobiotic-mediated induction of CYP2B6, CYP2C8/9, and CYP3A4 (24). In the present investigation, we used MED1 liver conditional null (MED1$^{\Delta Liv}$) mice to assess the development of hepatic steatosis caused by Dex treatment. The studies show that absence of *MED1/PPARPB* gene in liver markedly diminishes hepatic steatotic response in liver following Dex administration, implying that this coactivator is essential for GR and CAR functions.

MATERIALS AND METHODS

Animals and Treatment

Generation of mice carrying liver-specific MED1 ablation (MED1$^{\Delta Liv}$) has been described previously (8). Mice used in these studies were age matched (~5-week-old males; 5–8 mice for each time point) and maintained on a 12-h light/dark cycle. Dex, dissolved in corn oil, was administered IP at a dose of 50 mg/kg body weight for 1, 2, or 3 days. Mice injected with an equal volume of corn oil served as controls (27). All animal studies were approved by the Northwestern University Institutional Animal Care and Use Committee.

Livers were fixed in 10% formalin or 4% paraformaldehyde, and 4-μm-thick paraffin sections were cut and stained with hematoxylin and eosin. Additional sections were stained immunohistochemically with antibodies against MED1 [TRAP220 (C-19); Lot 20, Santa Cruz Biotechnology] as described elsewhere (8,17). Frozen sections of formalin-fixed liver (5 μm thick) were stained with Oil red O and counterstained with Giemsa.

TABLE 1
REAL-TIME POLYMERASE CHAIN REACTION PRIMERS

ID	Target	Forward	Reverse
1	Constitutive androstane receptor (Car)	GCCATGGCCCTCTTCTCCC	TCAGCCAGCAGGCCCATCAG
2	Cyp2b10	GAACTGCGGAAATCCCAGGGAG	TCCAGCAGGCGCAAGAACTGAC
3	Cyp3A11	CTGCATTCCTTGGCCACTCACC	TGACTGCATCCCGTGGCACAAC
4	IGFBP2	TGCACCCGCCACGAGCAC	GGGCCATCAGGTGGAAGCTGTC
5	Acyl-CoA synthetase 2	CGCTTGTGGAGCATTGTGGAC	GGTCAGCATATGGCCACCTG
6	Saa1	TGTTCACGAGGCTTTCCAAGG	CACTGCGGCCATGTCTGTTG
7	Glucokinase	TGGGCTTCACCTTCTCCTTCC	AGCCGGTGCCCACAATCATG
8	ATP-binding cassette C3	GGGTGAGATCGTCATTGATGG	CTCCAAGTCAATGGCAGCAGTG
9	Tissue inhibitor of metalloproteinase 4	TGGTGCAGAGGGAGAGCCTG	TCGGTACCAGCTGCAGATGC
10	Glucose-6-phosphatase	CGCTATCTCCAAGTGAATTACC	CAAAGAGATGATGCAGGACC
11	Thyroid hormone responsive SPOT14	ATCCCAAGAACTGCCTGCTGAC	TTTCAGCAGCGTTCTCAG
12	Solute carrier family 2	CCATCTTCATGTCGGTGGGAC	CGTAAGGCCCAAGGAAGTCC
13	Acyl-CoA thioesterase 1	GGGCATCACAGCTGCTGTGG	CGCTCTTCCAGTTGTGGTCG
14	p21	TGGCCTTGTCGCTGTCTTGCAC	GGGCTCCCGTGGGCACTTC
15	p55	AGATCCGGGATCAGCACCTTG	CTGCAACCACAGAACAAGTG

Northern and Immunoblotting Procedures

Total RNA was isolated from liver using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. For Northern blotting, total RNA was glyoxylated, separated on 0.8% agarose gel, transferred to nylon membrane, and hybridized with ^{32}P-labeled cDNA probes (8). For immunoblotting, total liver proteins were subjected to 10 % SDS-PAGE, transferred to nitrocellulose membranes, and immunoblotted with antibodies as previously described (8). Protein concentration was determined by using a protein assay kit (Bio-Rad).

Real-Time PCR Analysis

For quantitative analysis, reverse transcription was performed on 2 μg of total RNA in a reaction volume of 20 μl using Superscript III First Strand Synthesis System for RT-PCR (Invitrogen). Quantitative real-time PCR (qPCR) was carried out in triplicates using primer pairs (Table 1) and normalized with 18S ribosomal RNA. PCR was composed of 1 μl (100 pmol) of sense and antisense primers and 10 μl of 2× SYBR Green Supermix (Applied Biosystems) to make a final volume of 20 μl and performed by using the ABI 7300 (Applied Biosystems).

Microarray Hybridizations and Data Analysis

Total RNA isolated from wild-type and MED1$^{\Delta Liv}$ mouse livers, following 3-day Dex treatment, was used for reverse transcription and second-strand synthesis. The cDNA product was then used for preparing biotin-labeled cRNA, which was purified, fragmented, and hybridized to 430 2.0 arrays (Affymetrix). After hybridization, bound cRNA was fluorescently labeled using R-phycoerythrin streptavidin (Molecular Probes), and the fluorescence was intensified by the antibody amplification method as previously described (17,34).

RESULTS

MED1 Deficiency Diminishes Dex-Induced Hepatic Steatosis

Dex, given at 50 mg/kg body weight daily for 3 days, caused ~60% increase in liver weight in MED1$^{+/+}$ mice compared to MED1$^{\Delta Liv}$ mice (data not shown). In wild-type (MED1$^{+/+}$) mice, macrovesicular fatty change was evident in liver lobules with 1-day Dex treatment, which became prominent at 3 days (Fig. 1A–D). In contrast, in MED1$^{\Delta Liv}$ livers, Dex treatment caused no perceptible increase in hepatic

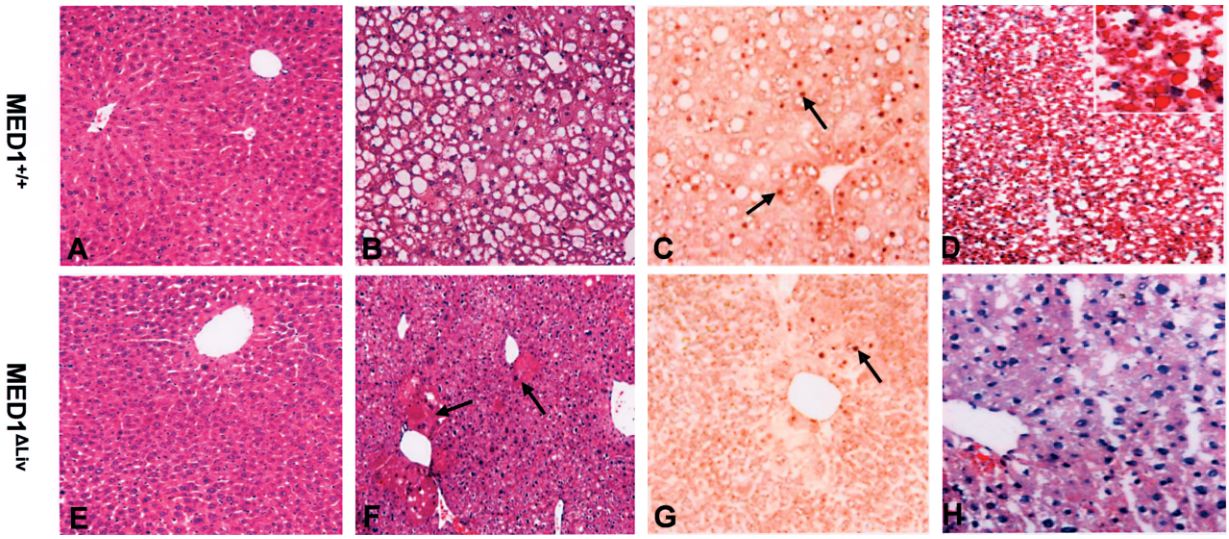

Figure 1. Histological analysis of liver from wild-type (MED1$^{+/+}$) and MED1$^{\Delta Liv}$ mice given Dex (50 mg/kg body weight, IP) or corn oil as vehicle once daily for 3 days. The liver sections of mice treated for 0 (A, E), and 3 days (B, F) were stained with hematoxylin and eosin. Liver sections from 3-day Dex-treated mice were processed for immunohistochemical localization of MED1 (C, G). Dex-treated MED1$^{+/+}$ mouse liver reveals prominent macrovesicular fatty change (B) and shows MED1 nuclear staining (C). Note the peripheral location of MED1-positive nucleus (arrows) due to displacement by large fat vacuole (C). Fatty change is minimal in MED1$^{\Delta Liv}$ mouse liver even at 3-day Dex treatment. An occasional MED1-positive nucleus is seen (G, arrow) due to escape from Cre-mediated deletion. Oil red O (D, H) staining of liver sections obtained from MED1$^{+/+}$ (D) and MED1$^{\Delta Liv}$ (H) mice treated with Dex for 3 days. Hepatic steatosis seen in Dex-treated MED1$^{+/+}$ mice with hematoxylin and eosin staining (B) is confirmed by Oil red O staining (D). In the livers of 3-day Dex-treated liver conditional knockout mouse an occasional large hepatocyte that escaped Cre-mediated deletion is present (F, arrows), which reveal MED1-positive nucleus in MED1$^{\Delta Liv}$ mouse liver (G, arrow).

steatosis at day 1, and only a minimal increase at 3 days (Fig. 1E–H). Oil red O staining of liver sections obtained from MED1$^{+/+}$ mice treated with Dex for 3 days confirmed hepatic lipid accumulation (Fig. 1D, H). Immunohistochemical staining with anti-MED1 antibodies revealed that all hepatocyte nuclei in wild-type mouse liver were stained positively for MED1 (Fig. 1C), but no nuclear staining was noted in MED1$^{\Delta Liv}$ livers (Fig. 1G). Due to large fat droplet accumulation in 3-day Dex treatment in wild-type hepatocytes, MED1-stained nuclei, which were pushed peripherally, gave these cells a typical signet ring appearance (Fig. 1C).

MED1 Deficiency Prevents Dex-Induced Reductions in Fatty Acid Oxidation Enzymes

Glucocorticoids inhibit mitochondrial fatty acid β-oxidation enzymes and hepatic lipid secretion (16). Dex has been shown to inhibit mitochondrial matrix located long-, medium-, and short-chain dehydrogenases (LCAD, MCAD, and SCAD, respectively) and this inhibition results in the impairment of mitochondrial fatty acid β-oxidation contributing to hepatic steatosis (16). The mechanism by which Dex, a ligand for nuclear receptor GR, inhibits these enzymes is unclear. It is possible that Dex could interfere with the functions of certain transcriptional cofactors and affect nuclear receptor function (12). We have investigated the role of coactivator MED1 in Dex-mediated inhibition of fatty acid metabolizing enzymes in the liver (16). Constitutive basal levels of expression of LCAD, MCAD, and SCAD that are involved in mitochondrial β-oxidation system appeared similar in MED1$^{+/+}$ and MED1$^{\Delta Liv}$ livers (Fig. 2A, B). Likewise, the levels of peroxisomal β-oxidation enzymes acyl-CoA oxidase-1 (ACOX1), enoyl-CoA hydratase/L-3-hydroxyacyl-CoA dehydrogenase (L-PBE), and 3-ketoacyl-CoA thiolase (PTL), and microsomal fatty acid oxidation enzyme CYP4A1 were also similar in both MED1$^{+/+}$ and MED1/PBP$^{\Delta Liv}$ livers (Fig. 2A, B). We show that the hepatic levels of mitochondrial, peroxisomal, and microsomal fatty acid oxidation enzymes LCAD, MCAD, SCAD, ACOX1, L-PBE, PTL, and CYP4A1 were inhibited by treatment with Dex for 3 days in wild-type mouse liver (Fig. 2). In

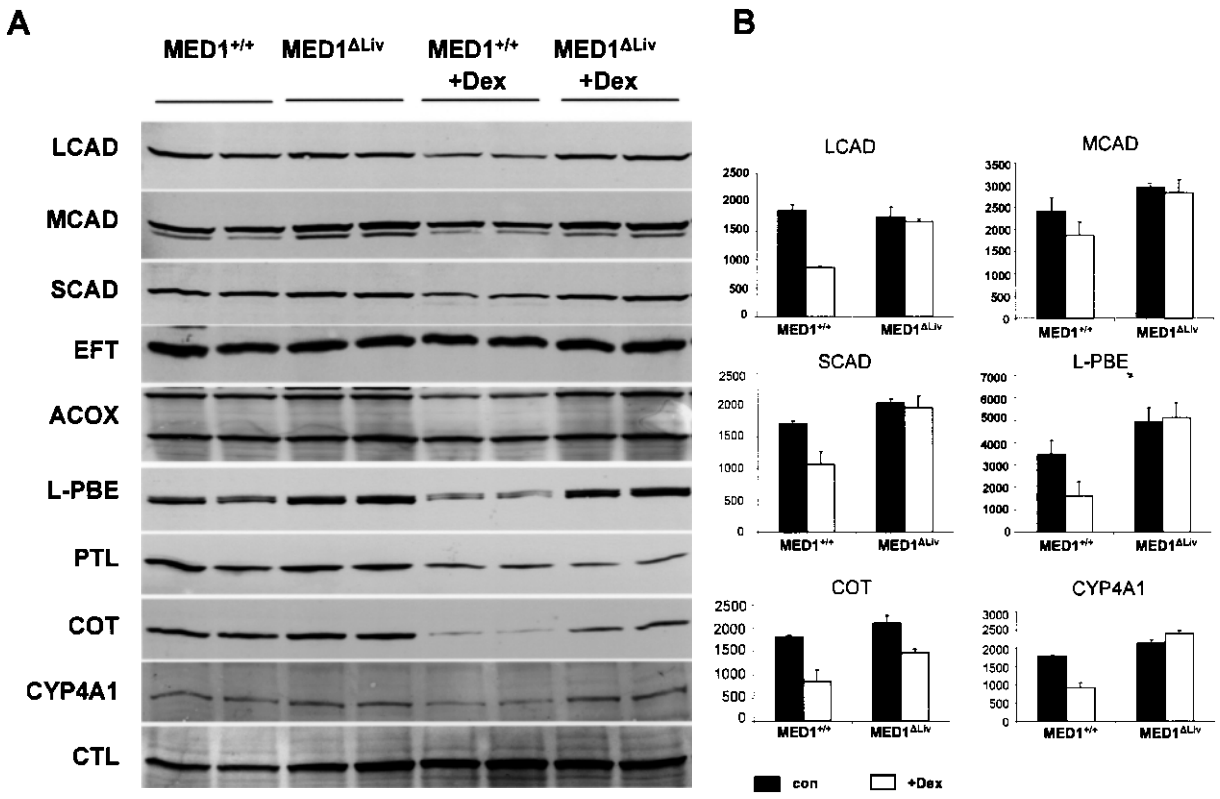

Figure 2. (A) Immunoblot analysis of liver proteins for changes in some enzymes responsible for fatty acid oxidation. LCAD, MCAD, and SCAD represent mitochondrial β-oxidation system enzymes, while ACOX, L-PBE, and PTL are members of peroxisomal β-oxidation pathway. CYP4A1 is a microsomal fatty acid β-oxidation system enzyme. Also included are EFT, COT, and CTL. (B) The histogram is the densitometric analysis of the Western blot signals. Black bars refer to wild-type (con) and white bars to Dex treatment (Dex) in MED1$^{+/+}$ and MED1$^{\Delta Liv}$ mice. All data are presented as the mean ± SD of three independent measurements.

MED1$^{\Delta Liv}$ mice, Dex treatment failed to inhibit the levels of these proteins in liver (Fig. 2A, B). Reduction in hepatic carnitine octanoyltransferase (COT) protein content was more marked in Dex-treated wild-type mouse liver compared to MED1$^{\Delta Liv}$ mouse livers (Fig. 2A, B). COT is involved in converting products of peroxisomal β-oxidation as substrates for mitochondrial β-oxidation (21). No reduction in electron transfer flavoprotein (ETF) and catalase (CTL) content was observed in wild-type and MED1$^{\Delta Liv}$ mice treated for 3 days with Dex (Fig. 2).

Reduction in CAR mRNA Level in MED1-Deficient Liver

Because nuclear receptor CAR is known to play a role in the pathogenesis of nonalcoholic steatohepatitis (33), it appeared necessary to ascertain hepatic CAR mRNA expression level in MED1$^{\Delta Liv}$ mice in response to GR ligand Dex (Fig. 3A). Disruption of *MED1/PPARBP* gene in liver resulted in a marked reduction in CAR mRNA expression but did not alter GR mRNA levels. Basal GR mRNA levels in liver were similar in wild-type and MED1$^{\Delta Liv}$ mice and these levels did not differ significantly after Dex treatment (Fig. 3). Real-time PCR data obtained from mice treated with Dex for 1, 2, or 3 days showed that Dex reduced CAR level slightly but transiently in wild-type liver after 2-day treatment but these levels recovered after 3-day Dex treatment. Dex administration did not affect the already low CAR mRNA levels in MED1$^{\Delta Liv}$ mouse livers (Fig. 3B).

Gene Expression Changes in MED1 Null Mice Following Dex Administration

To investigate further the role of MED1 in GR-regulated gene expression in the liver, we performed cDNA microarray analysis using liver RNA isolated from 3-day Dex-treated wild-type and MED1$^{\Delta Liv}$ mice. Biotin-labeled RNA probes from two groups were hybridized to Affymetrix 430 2.0 microarray chips containing 45,000 genes. Following Dex treatment, several genes were upregulated fourfold or higher in

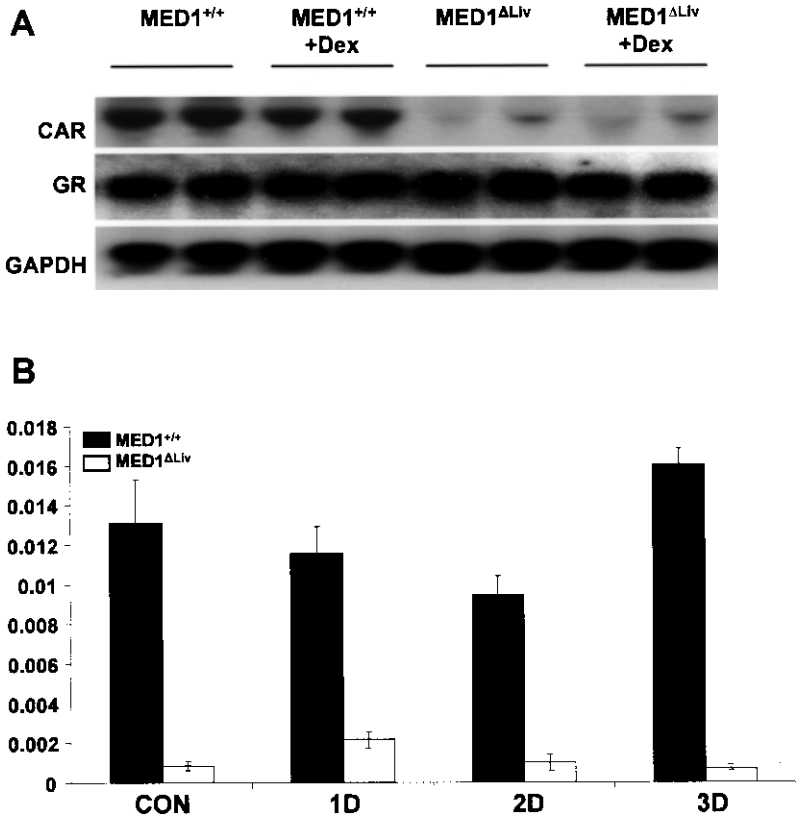

Figure 3. (A) Northern blot analysis for CAR and GR mRNA levels in MED1$^{+/+}$ and MED1$^{\Delta Liv}$ mouse livers without and with Dex treatment for 3 days. GAPDH is used as RNA loading control. (B) Quantitative real-time PCR analysis of CAR mRNA level in MED1$^{+/+}$ and MED1$^{\Delta Liv}$ mouse livers following treatment with Dex for 0 (con), 1 (1D), 2 (2D), and 3 (3D) days. Dark bars refer to wild-type (MED1$^{+/+}$) and white bars represent MED1$^{\Delta Liv}$ mice. All data are presented as the mean ± SD of three independent experiments.

TABLE 2
GENES UPREGULATED FOURFOLD OR GREATER IN DEX-TREATED WILD-TYPE (MED1$^{+/+}$) LIVER THAN IN MED1$^{\Delta Liv}$ LIVER

GenBank Accession	Fold Induction	Gene
Lipid metabolism genes		
NM_008295.1	233.0	Hydroxy-delta-5-steroid dehydrogenase, 3 beta
NM_007519	22.7	Bile acid-Coenzyme A: amino acid N-acyltransferase
NM_017399	16.8	Fatty acid binding protein 1 (Fabp1), liver
BC016468	11.0	Elongation of very long chain fatty acids 3
AK006128.1	10.7	ATP-binding cassette, sub-family C3
NM_008288.1	10.0	Hydroxysteroid 11-beta dehydrogenase 1(11β-HSD1)
BC019882.1	9.9	3-Ketoacyl-CoA thiolase B
NM_030611.1	9.7	Aldo-keto reductase family 1, member C6
BC027556	7.8	Lipocalin 13
NM_009381	6.7	Thyroid hormone responsive SPOT14 homolog (Rattus)
M63244	6.5	Aminolevulinic acid synthase 2, erythroid
NM_013821.1	5.9	Hydroxysteroid dehydrogenase-6, delta<5>-3-beta
NM_019811.1	5.8	Acyl-CoA synthetase short-chain family member 2
NM_019878.1	5.4	Sulfotransferase family 1B, member 1
BC026757.1	5.3	Hydroxysteroid dehydrogenase-2, delta<5>-3-beta
AV257512	5.2	Insulin induced gene 2
BC021836.1	5.1	Hydroxysteroid (17-beta) dehydrogenase 9
NM_008156.1	4.9	Glycosylphosphatidylinositol-specific phospholipase D1
NM_008280.1	4.9	Lipase, hepatic
NM_019811.1	4.5	Acyl-CoA synthetase short-chain family member 2
BB004104	4.4	Cytochrome P450, family 51
NM_133943.1	4.3	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7
NM_009512.1	4.1	Solute carrier family 27 (fatty acid transporter), member 5
Cell cycle, cell growth, and apoptosis genes		
NM_007634	28.9	Cyclin F
NM_008566	20.3	Cell division cycle 46 (S. cerevisiae)
NM_013808.1	14.0	Cysteine and glycine-rich protein 3
BC002031.1	11.9	BH3 interacting domain death agonist
NM_022032.1	10.1	TP53 apoptosis effector
NM_016719.1	9.0	Growth factor receptor bound protein 14
AV369812	8.7	Epidermal growth factor receptor
AK013765.1	6.6	Endothelial cell growth factor 1
AA796766	6.4	Metallothionein 2
AK011784.1	6.0	Insulin-like growth factor binding protein 2
NM_053176.1	5.8	Histidine-rich glycoprotein
AI256288	4.9	E2F transcription factor 8
AF275367.1	4.6	Epidermal growth factor receptor
AV252933	4.3	Growth arrest and DNA-damage-inducible 45 alpha
NM_011871.1	4.2	Protein kinase, interferon inducible double stranded RNA dependent activator
BC008997.1	4.0	Annexin A7
Glucose regulation genes and glycosylation		
BC011139.1	17.0	Glucokinase
BC021766.1	7.5	C-type lectin domain family 2, member h
NM_031197.1	6.5	Solute carrier family 2 (facilitated glucose transporter)
NM_133748.1	6.4	Insulin induced gene 2
AI265463	5.4	Phosphoenolpyruvate carboxykinase 1, cytosolic(PCK)
NM_028803.1	4.9	Glucan (1,4-alpha-), branching enzyme
AJ429133.1	4.9	Asparagine-linked glycosylation 12 homolog (yeast)
BB667395	4.7	Dehydrogenase E1 and transketolase domain containing 1
NM_008061	4.2	Glucose-6-phosphate
Signal transduction genes		
BB319311	18.9	Protein kinase, cGMP-dependent, type I
AW491150	11.2	MAP/microtubule affinity-regulating kinase 1
BB667397	10.7	Protein kinase C, nu
AV216412	8.0	Eukaryotic translation initiation factor 4E binding protein 1
NM_032400.1	7.4	Succinate receptor 1

(*continued*)

TABLE 2
CONTINUED

GenBank Accession	Fold Induction	Gene
BG075165	6.8	Insulin-like growth factor 1
NM_016847.1	6.4	Arginine vasopressin receptor 1A
NM_009647.1	5.7	Adenylate kinase 3 alpha-like 1
BI156474	4.9	Phosphatidylinositol 4-kinase type 2 beta
AK004874.1	4.2	Rap guanine nucleotide exchange factor (GEF) 4
Transcription/translation regulation genes		
NM_011082.1	23.6	Polymeric immunoglobulin receptor
C80642	21.5	Ankyrin repeat and IBR domain containing 1
BB458460	21.3	Coiled-coil-helix-coiled-coil-helix domain
BC010807.1	8.7	Transcription elongation factor A (SII), 3
BM239828	8.0	Interferon inducible GTPase 1
NM_009349.1	6.9	Indolethylamine N-methyltransferase
AI461691	6.2	Heat shock 70kDa protein 4 like
BF018652	5.5	SoxLZ/Sox6 leucine zipper binding protein in testis
BB183854	4.8	B-cell leukemia/lymphoma 6
BB305306	4.7	DEAD (Asp-Glu-Ala-Asp) box polypeptide 47
BQ177743	4.7	Wolf-Hirschhorn syndrome candidate 1 (human)
BG092043	4.6	Insulin-like growth factor 2, binding protein 3
D90176.1	4.5	Nuclear factor I/A
BB284697	4.1	Zinc finger protein 161
Extracellular matrix/cell structure, receptor, adhesion, and chaperone genes		
AV241307	65.4	Myomesin 2
NM_009255.1	24.5	Serine (or cysteine) peptidase inhibitor, clade E
BC015252.1	21.2	Claudin 2
AI447325	15.0	Rho GTPase activating protein 26
AK017358.1	12.0	Intergral membrane protein 1
NM_008645.1	11.9	Murinoglobulin 1
NM_019410.1	9.0	Profilin 2
AV227581	8.2	Claudin 1
NM_012050.1	5.8	Osteomodulin
AI461691	5.8	Heat shock 70-kDa protein 4 like
BG065575	5.7	Poliovirus receptor-related 1
NM_010746.1	5.2	Natural cytotoxicity triggering receptor 1
AK019164.1	4.9	Multiple PDZ domain protein 1
NM_133903.1	4.2	Spondin 2, extracellular matrix protein
Inflammation/immune-related genes		
BC027748.1	98.6	Complement component 8
NM_013485.1	24.2	Complement component 9
NM_009117	17.8	Serum amyloid A 1
NM_011314.1	16.4	Serum amyloid A 2
BC026555.1	13.8	Kallikrein B, plasma 1
BB794642	13.0	Melanoma antigen, 80 kDa
BC024380.1	12.0	Defensin beta 1
NM_017370.1	8.5	Haptoglobin
NM_007576.1	7.9	Complement component 4 binding protein
BI328146	7.3	Retinoic acid receptor responder (tazarotene induced)
NM_016704.1	6.0	Complement component 6
BC022123.1	4.9	Complement component 1, s subcomponent
BB667823	4.8	Ring finger protein 125
BE628912	4.7	Orosomucoid 1
Metabolic and other genes		
BF383739	143.3	Serine (or cysteine) peptidase inhibitor, clade A, 4
BC025936.1	134.8	Cytochrome P450, family 4, subfamily a, polypeptide 12
NM_023617.1	96.7	Aldehyde oxidase 3
NM_007825.1	79.3	Tissue inhibitor of metalloproteinase 4
BB328405	71.6	Cytochrome P450, family 7, subfamily b, polypeptide 1
NM_007860.1	59.2	Deiodinase, iodothyronine, type I

TABLE 2
CONTINUED

GenBank Accession	Fold Induction	Gene
BC010973.1	46.3	Cytochrome P450, family 8, subfamily b1
AK011413.1	39.4	Major urinary protein 1
BC026598.1	25.4	Solute carrier family 22, member 7
AI327006	23.5	Cytochrome P450, family 4, subfamily a14
NM_134144.1	22.7	Cytochrome P450, family 2, subfamily C50
NM_008030	20.0	Flavin containing monooxygenase 3
AK003671.1	19.0	Carbonic anhydrase 3
NM_021456.1	16.4	Carboxylesterase 1
NM_009993.1	12.2	Cytochrome P450, family 1, subfamily a2
NM_023135.1	12.0	Sulfotransferase family 1E, member 1
AA571276	11.5	Liver-expressed antimicrobial peptide 2
NM_009669.1	10.1	Amylase 2, pancreatic
NM_017473.1	10.0	Retinol dehydrogenase 7
L07645.1	9.6	Histidine ammonia lyase
NM_053215.1	9.3	UDP-glucuronosyltransferase 2 family, polypeptide B37
BC021378.1	9.2	NADPH oxidase 4
J03953.1	8.6	Glutathione S-transferase, mu 3
BC025819.1	8.5	Cytochrome P450, family 2, subfamily C44
BB293163	8.1	Mitochondrial ribosomal protein L30
AF128849.1	8.0	Cytochrome P450, family 2, subfamily b10
M63244.1	7.9	Aminolevulinic acid synthase 2, erythroid
AB021226	7.7	Matrix metallopeptidase 24
NM_007809.1	7.6	Cytochrome P450, family 17, subfamily a1
BC012682.1	7.3	Hydroxysteroid (17-beta) dehydrogenase 2
NM_011579.1	6.9	T-cell specific GTPase
AK013765.1	6.6	Endothelial cell growth factor 1
NM_007817.1	6.5	Cytochrome P450, family 2, subfamily F2
AB039380.1	6.2	Cytochrome P450, family 3, subfamily A44
NM_010403.1	5.5	Hydroxyacid oxidase 1, liver
BF783609	5.7	Cytochrome P450, family 2, subfamily j, polypeptide 5
NM_017396.1	4.8	Cytochrome P450, family 3, subfamily a, polypeptide 41
BB139766	4.8	Cytochrome P450, family 2, subfamily r, polypeptide 1
AI172943	4.5	Glutathione S-transferase, alpha 3
AV078914	4.3	Hydroxyacyl-Coenzyme A dehydrogenase type II
BC025940.1	4.3	UDP glycosyltransferases 3 family, polypeptide A1
NM_009676.1	4.2	Aldehyde oxidase 1
NM_011996.1	4.2	Alcohol dehydrogenase 4 (class I), pi polypeptide
Transport and storage genes		
M16359.1	215.3	Major urinary protein III (MUP III)
AB031813.1	95.3	Solute carrier organic anion transporter family 1
NM_007474.1	23.5	Aquaporin 8
BB553107	9.6	Solute carrier organic anion transporter family, 2b1
BC021154.1	8.7	Solute carrier family 10 (sodium/bile acid cotransporter family), member 1
NM_009205.1	6.2	Solute carrier family 3, member 1
AA276202	6.0	Solute carrier family 23 (nucleobase transporters)
NM_007752.1	5.0	Ceruloplasmin
AW208574	4.2	Lipopolysaccharide binding protein
NM_030687.1	4.0	Solute carrier organic anion transporter family, member 1a4
Miscellaneous		
BB795733	165.9	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase
NM_026822.1	5.9	Small proline rich-like 5
AK004030.1	4.9	Synaptogyrin 2
NM_029562.1	4.9	Cytochrome P450, family 2, subfamily d, polypeptide 26
NM_134069.1	4.6	Solute carrier family 17 (sodium phosphate), member 3
BC024104.1	4.6	Protein Z, vitamin K-dependent plasma glycoprotein
BC027200.1	4.5	UDP glucuronosyltransferase 2 family, polypeptide B1
NM_029550.1	4.3	Kidney expressed gene 1
NM_029796.1	4.2	Leucine-rich alpha-2-glycoprotein 1
AI876438	4.1	Ectonucleotide pyrophosphatase/phosphodiesterase 3
AU016334	4.0	Heterogeneous nuclear ribonucleoprotein A3
NM_012059.2	4.0	SH3 domain protein D19

TABLE 3
GENES UPREGULATED FOURFOLD OR GREATER IN DEX-TREATED MED1$^{\Delta Liv}$ LIVER COMPARED TO WILD-TYPE (MED1$^{+/+}$) LIVER

GenBank Accession	Fold Induction	Gene
Lipid metabolism genes		
NM_012006.1	10.8	Acyl-CoA thioesterase 1 (Acot1)
NM_134188.1	7.5	Acyl-CoA thioesterase 2 (Acot2)
AU022584	7.2	SMC6 structural maintenance of chromosomes 6-like 1
Cell cycle, cell growth, and apoptosis genes		
BB221402	15.4	Cell death-inducing DFFA-like effector c
NM_007669.1	9.6	Cyclin-dependent kinase inhibitor 1A (P21)
BB538325	8.1	Cyclin D1
U52193.1	7.7	Phosphatidylinositol 3-kinase
BE951810	7.0	Neurofibromatosis 2
BB814564	6.1	Transformation related protein 53 binding protein 2
NM_011361.1	5.6	Serum/glucocorticoid regulated kinase
AV227314	4.8	Cysteine rich transmembrane BMP regulator 1 (chordin like)
AI451482	4.6	Cyclin-dependent kinase 7
BG065754	4.2	Cyclin G1
BB398886	4.2	PRKC, apoptosis, WT1, regulator
BI328541	4.0	Kinesin family member 5B
Glucose regulation genes		
AV272221	7.7	Nuclear receptor interacting protein 1
AU040643	7.3	Asparaginase like 1
NM_008062.1	4.1	Glucose-6-phosphate dehydrogenase X-linked
Signal transduction genes		
BB034265	13.7	Regulator of G-protein signaling 2
AA414954	13.2	Phosphatidylinositol 3 kinase, regulatory subunit, polypeptide 3 (p55)
NM_013749.1	13.0	Tumor necrosis factor receptor superfamily, member 12a
AA590970	9.6	Huntingtin interacting protein 1 related
M68515.1	8.4	Ephrin type-A receptor 3
BG070037	8.4	Neuronal PAS domain protein 2
NM_139059.1	6.8	Casein kinase 1, delta
BC003906.1	5.7	Tumor necrosis factor, alpha-induced protein 1 (endothelial)
AI451642	5.5	B-cell scaffold protein with ankyrin repeats 1
AK005325.1	4.9	A kinase (PRKA) anchor protein 10
BC023022.1	4.8	Calcium binding atopy-related autoantigen 1
BF136544	4.8	Fibrinogen-like protein 2
AV025452	4.2	G protein-coupled receptor 135
D17444.1	4.1	Leukemia inhibitory factor receptor
Transcription genes		
AI506429	19.8	RIKEN cDNA 5830411E10 gene
NM_025705.1	17.9	Discoidin, CUB and LCCL domain containing 1
AV305197	15.8	Striatin, calmodulin binding protein 3 (Strn3)
NM_008539.1	13.4	MAD homolog 1 (Drosophila)
AI414736	12.1	Zinc finger, MYND domain containing 12
AV227804	10.0	U3 small nucleolar ribonucleoprotein, homolog (yeast)
BC028550.1	9.9	Histone 1, H4h
BB333374	9.5	Zinc finger protein 533
NM_013550.1	8.7	Histone 1, H3a
NM_133971.1	8.5	Ankyrin repeat domain 10
BM235074	8.0	Interleukin enhancer binding factor 2
BM245170	7.5	Fos-like antigen 2
AV012790	7.3	Splicing factor, arginine/serine-rich 12
NM_009637.1	6.6	AE binding protein 2
AV312048	6.3	Splicing factor, arginine/serine-rich 2, interacting protein
NM_023672	6.1	Single-stranded DNA binding protein 3
NM_025299.1	5.9	Thioredoxin-like 4
NM_016859.1	5.6	Bystin-like
BQ177107	5.3	Potassium channel tetramerisation domain containing 13
NM_009281.2	5.2	Zinc finger protein 143
AK018187.1	4.8	Zinc finger protein 518
BI690586	4.5	Thyroid hormone receptor associated protein 1

TABLE 3
CONTINUED

GenBank Accession	Fold Induction	Gene
BC016565.1	4.5	Peptidyl prolyl isomerase H
BB234363	4.4	Transmembrane protein 69
BC024613.1	4.4	Transmembrane protein 37
BQ179556	4.1	Mitochondrial ribosomal protein L17
AW985925	4.0	Transmembrane protein 23
W77144	4.0	Inhibitor of DNA binding 4
Extracellular matrix/cell structure genes		
BB667295	13.5	RIKEN cDNA 2310044D20 gene
NM_007598.1	6.7	CAP, adenylate cyclase-associated protein 1
NM_020568.1	5.5	Plasma membrane associated protein, S3-12
AV071536	4.6	Integrin alpha 1
NM_023662.1	4.5	Pericentriolar material 1
Inflammation-related genes		
NM_009846.1	11.9	CD24a antigen
NM_007572.1	5.6	Complement component 1, q subcomponent, alpha polypeptide
Metabolic and other genes		
AF192558.2	22.0	Mitochondrial carrier homolog 1 (C. elegans)
N28171	13.5	DNA segment, Chr 16, Brigham & Women's Genetics 1494 expressed
BC027445.1	9.7	Protein tyrosine phosphatase 4a3
AW763765	9.2	Heat shock protein 1A
AV023312	7.2	ADP-ribosylation factor 2
NM_011884.1	6.3	RNA guanylyltransferase and 5'-phosphatase
AV313469	5.6	Zinc finger, CSL-type containing 3
NM_021486.2	5.5	Beta-carotene 15,15'-monooxygenase
BM222742	5.5	PDZ and LIM domain 5
BB667459	5.0	Vmyotubularin related protein 12
NM_133705.1	4.9	Pyrroline-5-carboxylate reductase family, member 2
BG067251	4.9	O-sialoglycoprotein endopeptidase-like 1
AV317107	4.6	SUMO/sentrin specific peptidase 2
BC024135.1	4.6	Coenzyme Q6 homolog (yeast)
AV023994	4.3	Cathepsin L
NM_007423.1	4.2	Alpha fetoprotein
AA792094	4.1	Glutamate oxaloacetate transaminase 1, soluble
Transport and storage genes		
NM_011075.1	17.7	ATP-binding cassette, sub-family B (MDR/TAP), member 1B
NM_025960.1	10.0	Trafficking protein particle complex 6A
AV343478	8.3	ATPase, Ca++ transporting, plasma membrane 2
AY061807.1	6.7	Calmodulin-like 4
NM_025409	6.2	Immediate early response 3 interacting protein 1
BB497312	6.1	Solute carrier family 13, member 3
BM250411	5.9	Solute carrier family 39 (zinc transporter), member 10
NM_054055	4.8	Solute carrier family 13, member 3
BE686616	4.0	Mitochondrial translational release factor 1-like
Miscellaneous		
AI507307	12.9	Suprabasin
BB104669	12.2	Kinesin family member 2C
BG067897	12.0	Trinucleotide repeat containing 6b (Tnrc6b), transcript variant 1
C77631	10.7	Expressed sequence
BB021163	10.0	EST
BC008229.1	9.6	RIKEN cDNA 1500011J06 gene
BB102308	9.4	Expressed sequence AW228944
BC028777.1	9.3	RIKEN cDNA 1600002H07 gene
BC011230.1	9.2	RIKEN cDNA 2510015F01 gene
AK017143.1	9.2	RIKEN cDNA 5031425E22 gene
BM936291	8.9	RIKEN cDNA 2310047C04 gene
BB546429	8.8	EST
AV145060	8.7	Phosphofurin acidic cluster sorting protein 2
BB344827	8.4	PRP4 pre-mRNA processing factor 4 homolog B (yeast)
AI510297	8.4	RIKEN cDNA 2700007P21 gene

(*continued*)

TABLE 3
CONTINUED

GenBank Accession	Fold Induction	Gene
BM230508	8.2	RIKEN cDNA A030007D23 gene
NM_030697.1	8.2	Ankyrin repeat domain 47
BM932452	8.2	Short coiled-coil protein
BG071865	6.7	Serine/arginine repetitive matrix 1
AV369290	6.7	TBC1 domain family, member 5
AK017464.1	6.6	Sestrin 3
BC018474.1	6.1	EPM2A (laforin) interacting protein 1
AF265663.1	5.9	MLX interacting protein
BG069557	5.7	PRP3 pre-mRNA processing factor 3 homolog (yeast)
AV218922	5.7	RIKEN cDNA 2610002J02 gene
BB667703	5.6	RIKEN cDNA 2410127E18 gene
BF225441	5.4	RIKEN cDNA 2210010L05 gene
BI453712	5.2	Hematological and neurological expressed sequence 1
BI737178	5.2	RIKEN cDNA 2610005L07 gene
BE945468	5.1	RIKEN cDNA 9530057J20 gene
BB375402	5.1	cDNA sequence BC027663
BC027371.1	4.7	RIKEN cDNA 5830411E10 gene
NM_024452.1	4.6	Leucine zipper protein 1
BE457727	4.6	RIKEN cDNA 5830411E10 gene
AK006222.1	4.6	RIKEN cDNA 1700021P22 gene
BE980579	4.5	A disintegrin and metallopeptidase domain 17
BC021385.1	4.3	RIKEN cDNA 9030617O03 gene
AW558420	4.2	DNA segment, Chr 6, ERATO Doi 253, expressed
BF147713	4.0	Leucine rich repeat containing 39
BB752934	4.0	RIKEN cDNA 3300001M20 gene

wild-type mouse liver when compared to MED1$^{\Delta Liv}$ and these fall into diverse functional categories (Table 2). A majority of genes induced by Dex in wild-type mouse liver is involved in lipid and glucose metabolism (1,7,10,23,25). Genes involved in lipid metabolism that were increased greater than sixfold include hydroxysteroid dehydrogenase-3β, bile acid-coenzyme A, fatty acid binding protein 1, elongation of very long chain fatty acids, ATP-binding cassette subfamily 3, hydroxysteroid 11β-dehydrogenase 1 (11βHSD1) (13), lipocalin 13, and thyroid hormone responsive SPOT14 (2). Acyl-CoA synthetase, phosphoenoylpyruvate carboxykinase-1 (PEPCK1), hepatic lipase, glucose-6-phosphatase (G6Pase), and solute carrier family 27 fatty acid transporter, which also play a role in lipid and glucose metabolism, were also upregulated by Dex in wild-type mouse livers compared to livers lacking MED1. The expression of complement components 8 and 9, serum amyloid A1, A2, and kallikrein B, and others related to inflammatory and acute response processes was also sixfold or greater in MED1$^{+/+}$ livers compared to MED1$^{\Delta Liv}$ mouse livers treated with Dex. A significant correlation has been reported between the expression of inflammatory genes and liver triglyceride content (30). In wild-type mouse liver Dex treatment also caused an increase in serine peptidase inhibitor clade A4, several members of cytochrome P450 family, aldehyde oxidase, deiodinase iodothyronine type 1, tissue inhibitor of metalloproteinase 4 (TIMP4), and others. Several genes, such as acyl-CoA thioesterase 1, cell death-inducing DFFA-like effector C (>15-fold), cyclin-dependent kinase inhibitor 1A (p21), cyclin D1, phosphotidylinositol 3-kinase, and nucleic acid binding protein 1 (NABP1) (11), were upregulated in MED1$^{\Delta Liv}$ mouse liver following Dex treatment when compared to Dex-treated wild-type mouse (Table 3).

Validation of Gene Expression of Selected Genes With Quantitative PCR Analyses

From the highly expressed genes identified by microarray, using 3-day Dex-treated wild-type and MED1$^{\Delta Liv}$ mouse livers we selected several genes for validation by quantitative PCR analysis (Figs. 4 and 5). We used RNA from mice treated for 1, 2, and 3 days. The predicted high levels of expression of genes in wild-type mouse livers were confirmed by qPCR analysis. These include insulin-like growth factor binding protein 2 (IGFBP2) (22,36), glucokinase, G6Pase, thyroid hormone responsive SPOT14 (2), tissue inhibitor of metalloproteinase 4, Saa1, solute carrier family 2 (glucose transporter), member 2, ATP-binding cassette, C3 (abcc3), and others (Fig. 4). We also validated the predicted increases in the expression of genes identified by microarray in Dex-

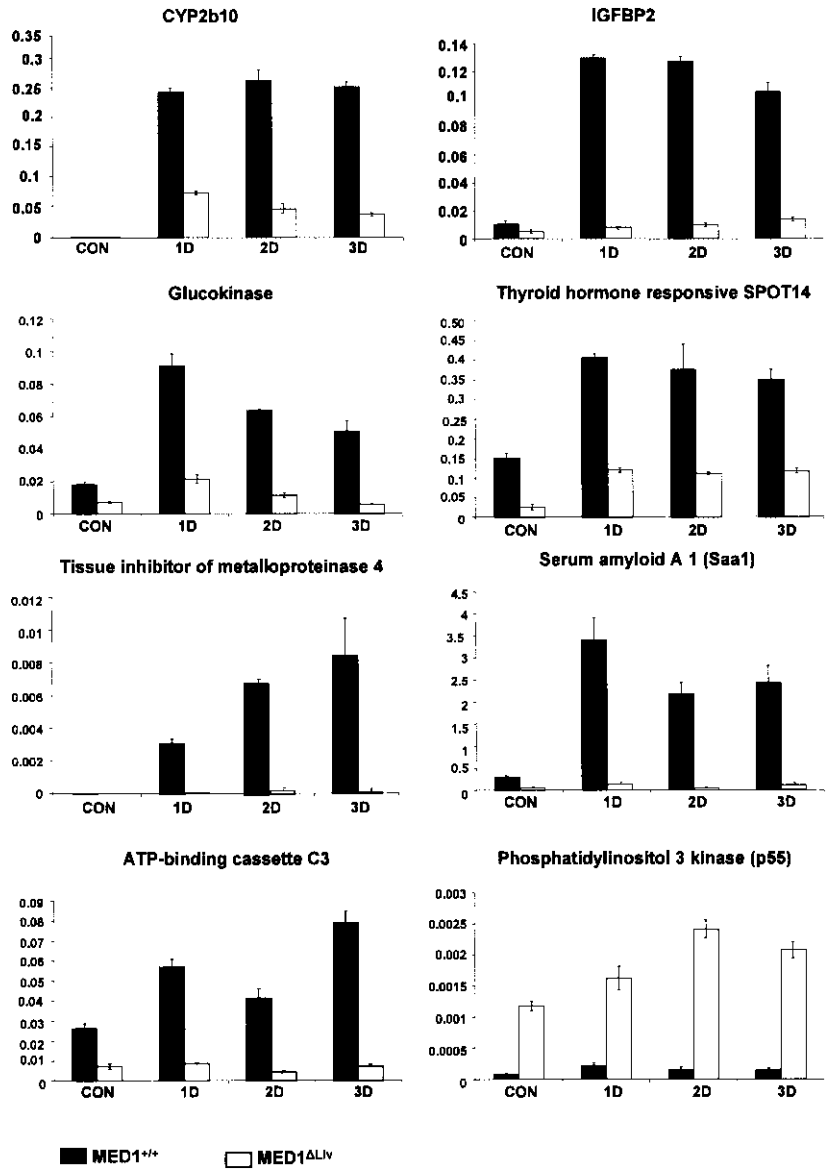

Figure 4. Comparative expression of genes in liver selected from microarray profile data in the livers of MED1$^{+/+}$ and MED1$^{\Delta Liv}$ mice after 1, 2, and 3 days of treatment with Dex. Increases in CYP2b10, IGFBP2, glucokinase, thyroid responsive SPOT14, tissue inhibitor of metalloproteinase 4, Saa1, and ATP-binding cassette C3 are seen in Dex-treated wild-type (black bars) compared to MED1$^{\Delta Liv}$ mice (white bars). The microarray predicted increase in phophatidylinositol 3 kinase (p55) in Dex-treated MED1$^{\Delta Liv}$ mouse liver is confirmed by quantitative PCR. The specific amplification of genes was normalized with 18S RNA signal and the arbitrary values are shown. All data are presented as the mean ± SD of three independent experiments.

treated MED1$^{\Delta Liv}$ mouse livers (Figs. 4, 5). These included acyl-CoA thioesterase 1, cyclin-dependent kinase inhibitor 1A (p21), and phosphatidylinositol 3 kinase (p55) (Figs. 4, 5).

DISCUSSION

Nonalcoholic fatty liver disease (NAFLD) results from an excessive accumulation of TGs in hepatic parenchymal cells emanating from a variety of pathophysiological perturbations. These include metabolic diseases such as obesity, type 2 diabetes, and conditions associated with chronic increases in glucocorticoid levels resulting in sustained activation of the GR, a transcription factor (31). The GR is present in the cytosol, which, upon glucocorticoid binding, translocates into the nucleus to serve as a transcriptional regulator of distinct sets of target genes to elicit a plethora of glucocorticoid responses (10,12). Like other transcription factors, GR recruits several cofactors, including MED1, a pivotal subunit of mediator

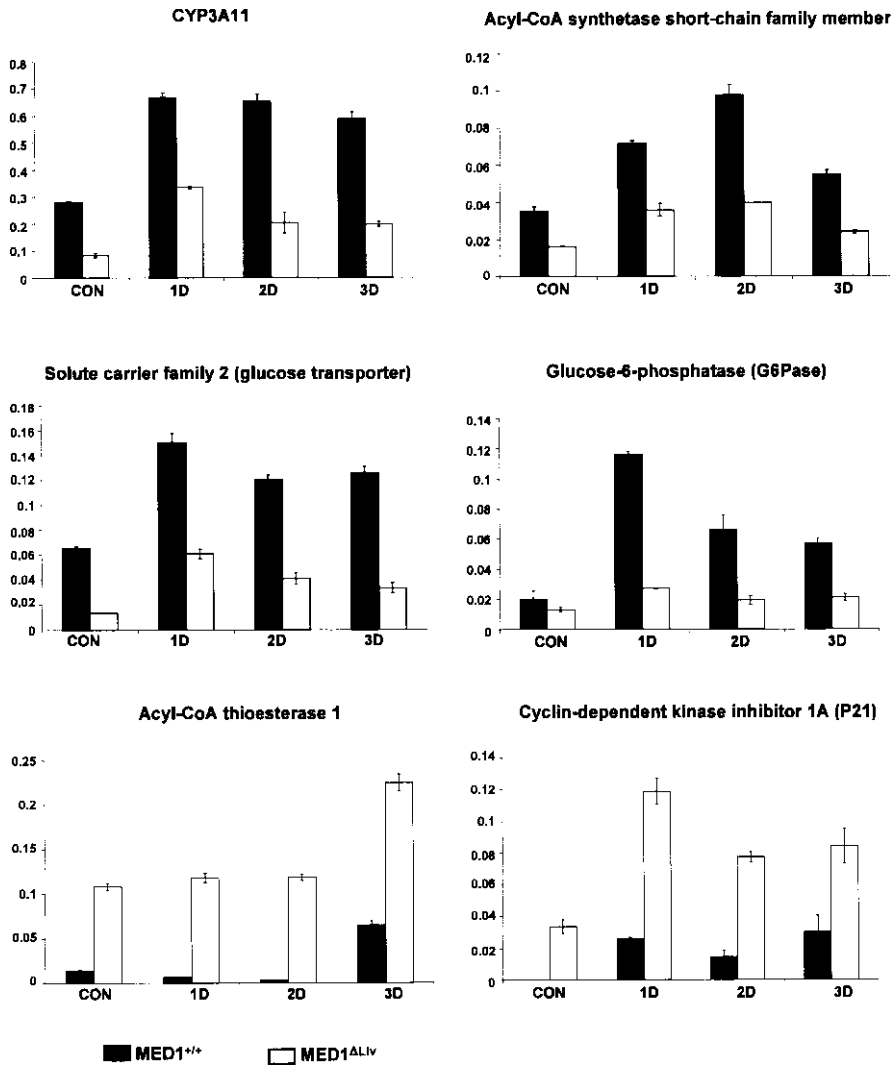

Figure 5. Further validation of microarray findings by quantitative PCR of hepatic RNA of MED1$^{+/+}$ and MED1$^{\Delta Liv}$ mice after 1, 2, and 3 days of treatment with Dex. Increases in CYP3A11, acyl-CoA synthetase, glucose transporter, glucose-6-phosphatase (G6pc) are seen in Dex-treated wild-type (black bars) compared to MED1$^{\Delta Liv}$ mice (white bars). On the other hand, increases in acyl-CoA thioesterase 1 and cyclin-dependent kinase inhibitor (p21) are observed in Dex-treated MED1$^{\Delta Liv}$ mouse liver. The specific amplification of genes was normalized with 18S RNA signal and the arbitrary values are shown. Data are shown as the mean ± SD of three independent experiments.

complex, for optimal transcriptional activation (3,4,8, 9). Whole-body MED1 knockout mice are not viable, demonstrating the critical importance of this coactivator for proper functioning of many genes for survival (6,38). In this study, we used conditional gene disruption approach to show that the absence of MED1 in mouse liver abrogates GR agonist Dex-induced hepatic steatosis. In the liver, GR is a critical regulator of lipid and glucose homeostasis (15,26,32). Multiple genes are either repressed or activated during Dex-mediated GR activation, which determines overall energy homeostasis (10,26,32). In particular, Dex-induced fatty liver development is attributed to both increased TG synthesis in the liver, due in part to the induction of acetyl-CoA-carboxylase and fatty acid synthase, the key lipogenic enzymes (15,32), and to decrease in mitochondrial fatty acid β-oxidation by molecular mechanisms that remain largely elusive (16).

Dex activation of the GR has been shown to decrease hepatic expression of the cAMP-inducible transcriptional repressor hairy enhancer of split1 (Hes1) and this reduction appears to be a common feature of hepatic steatosis (15). Liver-specific knockdown of the GR expression has been shown to improve steatotic phenotype due to Hes1 overexpression, which represses or downregulates PPARα and its downstream regulator FSP27 and limits liver lipid accumulation (15,18,34). Our microarray data revealed approximately twofold increase in Hes1 mRNA level in

liver of MED1$^{\Delta Liv}$ mice when compared to wild-type mouse liver following Dex treatment. Thus, the effect of MED1 deficiency in liver appears similar to that observed with GR knockdown in liver. Increases in Hes1 level in GR and MED1-deficient livers could repress PPARα and its downstream lipogenic genes, resulting in the reduction of hepatic steatosis induced by Dex.

The results of this study with MED1$^{\Delta Liv}$ mice suggest that MED1 is required for the repression as well as activation of GR-regulated genes in the liver. For example, absence of MED1 reverses Dex-mediated repression of fatty acid oxidation. As a consequence, energy burning remains unaffected in Dex-treated MED1$^{\Delta Liv}$ mice, thus abrogating the development of hepatic steatosis. Dex and other glucocorticoids induce lipogenic enzymes such as fatty acid synthase, acetyl-CoA carboxylase, and 11βHSD1 in the liver, which add to hepatic lipid burden (32). These lipogenic genes were not induced in MED1$^{\Delta Liv}$ liver, suggesting that MED1 is needed for GR-mediated transcriptional activation of these genes. Glucocorticoids enhance hepatic gluconeogenic capacity by upregulating PEPCK transcript levels but, as seen in the present studies, PEPCK is not induced in the liver in the absence of MED1. PEPCK gene promoter has GR recognition elements and receptor occupation of these GREs results in the recruitment of cofactors such as PGC-1, SRC-1, and CBP/p300 for optimal transcriptional activation (32). The failure of the induction of lipogenic and gluconeogenic genes in MED1-deficient livers suggests that MED1 is critical for GR transcriptional activity. MED1 has been shown to interact with GR in a ligand-dependent manner (3,4). These studies also show that the absence of MED1 in the liver affects the induction of many genes involved in drug metabolism, possibly by influencing the transcriptional functions of nuclear receptors GR, CAR, PXR, and others (10,12,25). Using MED1 liver conditional null mice we establish that MED1 is required for in vivo function of GR. Our earlier work has also shown the pivotal role of MED1 in PPARα and CAR function in liver (5,8,9,17). It should be noted that PXR and CAR expression is glucocorticoid dependent (25) and a functional GRE has been identified in the CAR gene promoter (24). Previously, work from our laboratory established that MED1 is critical for CAR function in the liver and the data presented here show that CAR levels are markedly reduced in liver in the absence of MED1, clearly establishing that MED1 is need for GR-CAR signal transmission. CAR, like GR, is a cytoplasmic receptor, which when activated by a ligand translocates to the nucleus and that translocation is MED1 dependent (5,9). Whether GR translocation to the nucleus in the liver is MED1 dependent remains to be established. These studies clearly establish the importance of using tissue-specific deletion of MED1 to study the role of this coregulator in gene expression.

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