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Title Page

Overlapping Transcriptional Programs Regulated by the Nuclear Receptors Peroxisome Proliferator-Activated Receptor α, Retinoid X Receptor and Liver X Receptor in Mouse Liver

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Running Title Page

Running title: PPARα, RXR and LXR Regulate Overlapping Genes

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ABBREVIATIONS: ACO, acyl-CoA oxidase; BER, base excision repair; CAR, constitutive androstane receptor; FXR, farnesoid X receptor; LXR, liver X receptor; NR, nuclear receptor; PAGE, polyacrylamide gel electrophoresis; PBST, phosphate buffered saline plus Tween; PCR, polymerase chain reaction; PP, peroxisome proliferator; PPAR, peroxisome proliferator-activated receptor; PXR, pregnane X receptor; RT, reverse-transcriptase; RXR, retinoid X receptor; SDS, sodium dodecyl sulfate; WY, WY-14,643.

ABSTRACT

Lipid homeostasis is controlled in part by the nuclear receptors peroxisome proliferator (PP)-activated receptor α (PPARα) and liver X receptor (LXR) through regulation of genes involved in fatty acid and cholesterol metabolism. Exposure to agonists of retinoid X receptor (RXR), the obligate heterodimer partner of PPARα and LXR results in responses that partially overlap with those of PP. To better understand the gene networks regulated by these NR, transcript profiles were generated from the livers of wild-type and PPARα-null mice exposed to the RXR pan-agonist AGN194,204 or the PPAR pan-agonist, WY-14,643 (WY) and compared to the profiles from the livers of wild-type and LXRα/LXRβ-null mice after exposure to the LXR agonist T0901317. All 218 WY-regulated genes altered in wild-type mice required PPARα. Remarkably, ~80% of genes regulated by AGN194,204 required PPARα including cell-cycle genes, consistent with AGN-induced hepatocyte proliferation having both PPARα–dependent and –independent components. Overlaps of ~31-62% in the transcript profiles of WY, AGN194,204 and T0901317 required PPARα and LXRα/LXRβ for statistical significance. Out of the 50 overlapping genes regulated by T0901317 and WY, all but one were regulated in a similar direction. These results 1) identify new transcriptional targets of PPARα and RXR important in regulating lipid metabolism and liver homeostasis, 2) illustrate the importance of PPARα in regulation of gene expression by a prototypical PP and by an RXR agonist and 3) provide support for an axis of PPARα-RXR-LXR in which agonists for each nuclear receptor regulate an overlapping set of genes in the mouse liver.

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Introduction

Nuclear receptors (NR) are critical modulators of developmental and physiological processes and are both targets of drugs as well as chemicals of environmental significance. The NR peroxisome proliferator-activated receptor (PPARα,β,γ) subtypes are activated by a structurally diverse group of chemicals including peroxisome proliferators (PP) that increase the number and size of peroxisome organelles in mouse and rat liver (Klaunig et al., 2003). The PPAR subtypes have unique ligand-specificities (Corton et al., 2000) as well as expression patterns in the liver with PPARα expressed primarily in hepatocytes, PPARβ expressed in multiple cell types and PPARγ expressed in Kupffer cells (Braissant et al., 1996). In mice and rats, PPARα agonists elicit a predictable course of adaptive responses in the liver, including peroxisome proliferation, induction of lipid-metabolizing genes, and hepatomegaly (Klaunig et al., 2003). PPARα agonists have been used clinically for many years because of their ability to lower cholesterol and triglyceride levels in patients at risk for coronary heart disease (Hihi et al., 2002). Studies in wild-type and PPARα-null mice demonstrated that the phenotypic effects of PP exposure in the liver including peroxisome proliferation, cell proliferation and alteration of fatty acid metabolism genes depend on a functional PPARα (Lee et al., 1995; Klaunig et al., 2003). Although there is a general assumption that genes altered by PP in the liver are regulated by PPARα, a number of PP including WY-14,643 (WY) can act as PPAR pan-agonists activating PPARβ and γ in trans-activation assays (Kliewer et al., 1994). Notably, the PP bezafibrate can alter gene regulation in the livers of PPARα-null mice through PPARβ (Peters et al., 2003).

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Comprehensive transcript profiling in PP-treated wild-type and PPARα-null mice will be useful to determine the extent of PPARα-independent gene changes upon PP exposure.

The three retinoid X receptors (RXRα,β,γ) bind to the naturally occurring vitamin A derivative, 9-*cis* retinoic acid and are the targets of experimental drugs known as rexinoids. Rexinoids act as insulin sensitizers, and were beneficial in treating non-insulin-dependent (type 2, or adult-onset) diabetes and obesity in experimental models (Faul and Grese, 2002). The exact mechanisms by which RXR agonists modulate these responses have not been identified, partly due to the complexity of the interactions between RXR and NR. In addition to binding DNA response elements as a homodimer, RXR is required as a heterodimeric partner for a large number of NR (Rastinejad, 2001). These NR collectively called the Class II receptors include all PPAR subtypes as well as other receptors expressed in the liver including the liver X receptor. This promiscuity of RXR makes it difficult to separate the effects of RXR activation alone from activation through its heterodimeric partners. If RXR is to be exploited as a drug target, there is a need to understand the involvement of the class II receptors in the regulation of rexinoid gene targets.

Liver X receptors are key regulators of cholesterol metabolism. The two subtypes, LXRα and LXRβ, are activated by oxysterols and synthetic compounds including T0901317 (T1317) to regulate genes involved in cholesterol transport and metabolism to bile acids (Steffensen and Gustafsson, 2004; Tontonoz and Mangelsdorf, 2003). LXR also controls the expression of sterol regulator element-binding protein (SREBP)-1c, which regulates several lipogenic enzymes. Additional gene targets regulated by LXR include those involved in steroid hormone synthesis, growth hormone signaling and inflammation (Stulnig et al., 2002; Joseph et al., 2003).

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Nuclear receptors can "cross-talk" to ensure that antagonistic pathways are not simultaneously activated. For the class II receptors, cross-talk can occur through competition for a limiting amount of RXR so that activation of one NR-RXR heterodimer may have negative effects on the ability of other NR to heterodimerize with RXR and regulate gene expression. PPARα can antagonize the ability of LXR to activate the SREBP-1c promoter by titration of a limiting amount of shared RXR (Yoshikawa et al., 2003). Likewise LXR can antagonize the ability of PPARα to activate gene expression and this may occur through titration of RXR or through direct interactions between PPAR and LXR (Miyata et al., 1996; Ide et al., 2003). Comprehensive transcript profiles elicited by T1317 (Stulnig et al., 2002), PP and rexinoids would help to characterize any cross-talk between these receptors.

The objectives of the present study were to 1) determine the role of PPARα in mediating the transcriptional response to a PP, 2) classify PPARα-dependent and –independent targets of an RXR agonist and 3) determine the extent of the overlap in the transcriptional programs regulated by PPARα, RXR and LXR in the mouse liver.

Materials and Methods

Animal Treatments. All animal studies were conducted under federal guidelines for the use and care of laboratory animals and were approved by the Institutional Animal Care and Use Committee of the CIIT Centers for Health Research. Male wild-type and PPARα-null mice on a mixed SV129/C57BL/6 background were used in these studies and have been previously described (Lee et al., 1995). Mice were provided with NIH-07 rodent chow (Zeigler Brothers, Gardeners, PA) and deionized, filtered water *ad libitum.* Lighting was on a 12-h light/dark cycle. Mice received a single daily gavage dose between 9:00-10:00 AM of either the RXR pan-agonist

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AGN-194,204 (3 mg/kg/day, Allergan, Irvine, CA), the PPAR pan-agonist WY-14,643 (50 mg/kg/day, ChemSyn Science, Lenexa, KS) or the carrier methylcellulose (0.1%) each day for three days. These doses were selected based on previous studies showing that maximal transcriptional responses are induced in the absence of overt toxicity (Chandraratna, unpublished observations; Anderson et al., unpublished observations). Twenty-four hrs after the last dose, animals were deeply anesthetized with pentobarbital injection and killed by exsanguination. Portions of the liver were snap frozen in liquid nitrogen and stored at -70°C until processed for analysis of mRNA. Sections of liver were fixed in 10% neutral buffered formalin for 48 hrs, transferred to 70% ethanol, and embedded in paraffin. Subsequently, 5μm sections were mounted on slides, stained with hemotoxylin and eosin and examined by light microscopy.

RNA Isolation and Analysis of Gene Expression Using Oligonucletide Arrays. Three mice were analyzed from each of six treatment groups, for a total of 18 analyses. Hepatic RNA was isolated using a modified guanidium isothiocyanate method (TRIzol®, Invitrogen™, Carlsbad, CA) and was further purified using silica membrane spin columns (RNEasy® Total RNA Kit, Qiagen, Valencia, CA). RNA integrity was assessed by ethidium bromide staining followed by resolution on denaturing agarose gels and also by the RNA 6000 LabChip® Kit using a 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). For each sample from 18 individual mice, 15 μg of biotin-labeled cRNA was generated from 10 μg total RNA and hybridized to GeneChip® Test3 Arrays (Affymetrix, Inc., Santa Clara, CA) to determine quality. Subsequently, the same samples were hybridized to Murine GeneChip® U74Av2 oligonucleotide

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arrays (Affymetrix). All procedures were carried out according to the manufacturer's recommendations, using the antibody amplification technique. Images were initially processed using the MAS 5.0 software (Affymetrix). Hybridization quality was assessed by visual inspection of the image and from a report generated by MAS 5.0. Criteria for an acceptable hybridization were as follows: background < 100, noise (RawQ) < 5, 3'/5' ratio for select housekeeping genes < 4. Hybridizations not meeting these criteria were repeated, beginning at the target preparation step. The data were analyzed and statistically filtered using Rosetta Resolver® version 3.0 software (Rosetta Inpharmatics, Kirkland, WA). The threshold for significance was set at $p \leq 0.001$ and genes which exhibited a ≥1.5-fold or ≤-1.5-fold change were reported as a fold-change relative to the corresponding control. Genes altered by WY or AGN in wild-type and PPARα-null mice are reported. Sixty-four of these genes were also altered in control PPARα-null mice compared to control wild-type mice. An additional 59 genes were altered only in control PPARα-null mice compared to wild-type mice and will be characterized elsewhere. Similarly regulated genes were visualized using CLUSTER and TreeView (Eisen et al., 1998).

We compared the genes regulated by WY and AGN with those regulated by other Class II nuclear receptors in the liver. An initial analysis of available published gene lists for CAR, LXR, PXR, FXR and thyroid hormone receptor indicated that only the genes regulated by LXR exhibited significant overlap. We next determined the extent of the overlap directly by comparing the WY and AGN data set to that generated in wild-type and LXRα/LXRβ-null mice treated with the LXR agonist T1317 using the Affymetrix U74Av2 mouse chips (Stulnig et al., 2002). Genes that were reported as significant ($p \leq 0.001$) and exhibited a ≥1.5-fold or ≤-1.5-fold change were compared using CLUSTER and TreeView. Genes were grouped into functional

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classes with the help of KEGG (<http://www.kegg.org>) and using Gene Ontology (<http://www.geneontology.org>) identifiers in the U74Av2 template (version accessed Aug. 1, 2003 from http://www.affymetrix.com). Identification of ESTs was facilitated by euGenes (<http://iubio.bio.indiana.edu:89/mouse/>).

Real-time PCR Analysis of Gene Expression. Real-time PCR was performed as follows. After DNAse treatment, total RNA was quantified (Ribogreen®, Molecular Probes, Inc., Eugene, OR) and diluted with water. Fifty ng RNA and PCR reagents were aliquoted into 96-well plates using an ABI Prism™ 6700 Automated Nucleic Acid Workstation (Applied Biosystems, Foster City, CA) and subjected to real-time PCR (TaqMan®, Applied Biosystems) using gene-specific primers and fluorescently-labeled probes (Molecular Probes) (Supplementary Table 1) designed by the Primer Express® software (Applied Biosystems). Amplification curves were generated using the ABI Prism™ 7900HT Sequence Detection System (Applied Biosystems). Expression relative to vehicle control animals was determined after normalizing to the ribosomal *18S* gene (Applied Biosystems). There were 3 animals per treatment group and each sample was analyzed in duplicate. Variability is expressed as standard error of the mean.

Western Blot. Liver lysates were prepared in 250mM sucrose, 10mM Tris-HCl, pH 7.4, 1mM EDTA with protease inhibitors (0.2mM PMSF, 0.1% aprotinin, 1µg/ml pepstatin, 1µg/ml leupeptin) as previously described (Fan et al., 2003). Fifty µg of whole cell lysate was subjected to 12% SDS-PAGE followed by transfer to nitrocellulose membranes. Immunoblots were developed using primary antibodies against acyl-CoA oxidase (ACO) (a gift from Dr. S.

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Alexson, Huddinge University Hospital, Huddinge, Sweden), MFP-II and thiolase (gifts from Dr. Hashimoto, Japan), or Cyp4A (GenTest, Waltham, MA) and appropriate secondary antibodies conjugated with HRP (Santa Cruz Biotechnology, Santa Cruz, CA) in the presence of chemiluminescent substrate ECL (Amersham Biosciences, Piscataway, NJ). Blots were quantitated using Gel-Pro (MediaCybernetics, Silver Spring, MD).

Determination of Hepatocellular Proliferation. Osmotic minipumps (Alzet model 2001, 7 day pumps, 1 uL/h, Alza Corporation, Palo Alto, CA) filled with 16 mg/mL 5-bromo-2′-deoxyuridine (BrdU) in phosphate-buffered saline were implanted into the mice the day before the start of treatment. Nuclei that incorporated BrdU were identified by immunohistochemistry (Miller et al., 2000). Light microscopy was performed using a Microphot microscope (Nikon, Melville, NY) with a Dage CCD color video camera (DAGE-MTI, Inc., Michigan City, IN). The hepatocytes were analyzed using the Cytology Histology Recognition Identification System (CHRIS, Sverdrup Medical/Life Sciences Imaging Systems, Ft. Walton Beach, Fla). At least 1000 cells were counted for each animal. Cells that incorporated BrdU were identified by red pigmented nuclei. Ten to fifteen fields were counted. The labeling indices of hepatocytes were determined in different zones as previously described (Bahnemann and Mellert, 1997). To guarantee lobule comparison, the distance from the portal tract to the central vein was determined. Each lobule was subdivided into three parts, representing the three zones: zone 1 = periportal; zone 2 = intermediary; and zone 3 = perivenous. The labeling index (LI) was calculated by dividing the number of labeled hepatocyte nuclei by the total number of hepatocyte nuclei counted, and the results expressed as a percentage.

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Statistical Analysis of Data. Statistical test of significance was done by analysis of variance (ANOVA) post-hoc testing performed using the Tukey-Kramer test with a p-value of ≤ 0.05 (JMP™, SAS Institute, Research Triangle Park, NC). Spearman rank correlation test was performed using SAS (SAS v6.12, SAS Institute, Research Triangle Park, NC).

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Results

Transcriptional Programs Regulated by Agonists of PPARα and RXR. We identified 383 genes that were significantly ($p \leq 0.001$; ≥1.5-fold or ≤-1.5-fold change) different between two or more groups as outlined in the Materials and Methods. The identified genes were classified into major groups based on their expression behavior (Fig. 1). The first three classes were dependent on PPARα for altered expression after exposure. The largest class of genes (Class I) were regulated solely by WY. Class II genes were regulated by both WY and AGN. The regulation of these genes by AGN was not due to direct activation of PPARα, as AGN does not activate any of the PPAR subtypes (Chandraratna, unpublished observations). Class III genes were solely regulated by AGN. The Class IV genes, which were predominantly up-regulated, were altered by AGN in a PPARα-independent manner as regulation was approximately the same in both wild-type and PPARα-null mice. Class V genes were regulated by AGN only in PPARα-null mice. Class VI genes were defined as regulated by WY in a PPARα-dependent manner and AGN in a PPARα-independent manner. Class VII genes consisted of genes altered by WY only in PPARα-null mice. Class VIII genes were regulated by either WY or AGN in PPARα-null mice only. Class IX genes had mixed regulation. The classes I–IX contained 174, 31, 79, 18, 34, 9, 28, 5 and 5 genes, respectively. In addition, we identified genes that were dependent on PPARα for basal expression (discussed below) reinforcing the concept that PPARα controls the expression of many genes through activation by endogenous lipids including fatty acids (Aoyama et al., 2000).

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Two major conclusions can be drawn from the profiles. First, PPARα controls the vast majority of changes altered by WY exposure. Using our standard filtering criteria ($p \leq 0.001$; ≥1.5-fold or ≤-1.5-fold change), all 219 genes regulated by WY in wild-type mice were PPARα-dependent, i.e., similar expression was not observed in PPARα-null mice. Under these selection conditions however, there were 5 genes that exhibited opposite regulation by WY in PPARα-null mice vs. wild-type mice. If WY was activating PPARβ and PPARγ in PPARα-null mice, we would expect any changes to be relatively subtle due to dilution of these transcripts from PPARβ- or PPARγ–containing cell types by hepatocyte transcripts. We therefore repeated the selection of genes in the absence of a fold-change cut-off. Eleven genes were identified that exhibited similar regulation in the two strains (Supplementary Table 2) and 25 genes exhibited opposite regulation in the two strains (Supplementary Table 3). In this second group of genes, there was a significant negative correlation between gene expression in each strain ($R = -0.95$; $p < 0.001$ by Spearman analysis) as the fold-change rank order was roughly opposite in each strain.

The profiles also revealed that AGN required PPARα for the majority of gene expression changes. Using the standard filtering criteria, 111 out of a total 138 AGN-regulated genes (80.4%) were dependent on PPARα as expression changes after AGN treatment were observed in wild-type but not PPARα-null mice. To ensure that our selection criteria weren't biasing our results, we decreased the stringency of the cut-offs. When we removed the fold-change cut-off but retained the significance cut-off, more AGN-regulated genes were observed (372) but the percentage of those genes that required PPARα (303) remained approximately the same (81.5%). When we relaxed our filtering criteria to include only a $p < 0.05$ as a cut-off, 76.8% of

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the genes (602 out of 784) were PPARα-dependent. These findings indicate that PPARα plays a crucial role for an RXR agonist to elicit a transcriptional response in the mouse liver.

Overlap in the genes regulated by WY, AGN, and an LXR agonist. Our gene expression profiles identified genes regulated by AGN that were PPARα-independent (Class IV, V, VI, VIII, IX) indicating that AGN alters gene expression through other nuclear receptor-RXR heterodimers. To determine if there was an overlap in the transcript profiles determined by PPARα, RXR and LXR in the mouse liver, the profiles of WY and AGN were compared to those of the LXR agonist, T1317 in wild-type and LXRα/LXRβ-null mice as described in the Materials and Methods. All genes were filtered using the same criteria ($p \leq 0.001$; ≥1.5-fold or ≤-1.5-fold change). To determine the statistical significance of any overlapping genes, we performed a Spearman's rank correlation test on the 499 genes regulated by at least one of the 3 compounds in both wild-type and nullizygous mice (Table 1). There was a significant negative correlation in the 5 genes regulated by WY in wild-type versus PPARα-null mice, as discussed above. Significant overlaps existed between WY in wild-type mice and AGN in wild-type but not PPARα-null mice. Significant overlaps were also observed between WY and T1317 in wild-type but not in nullizygous mice, indicating that the overlap between WY and T1317 regulated genes requires PPARα and LXRα/LXRβ. The transcript profile of AGN in wild-type or PPARα-null mice had a significant overlap with T1317 in wild-type but not LXRα/LXRβ-null mice pointing to the possibility that some of the PPARα-independent genes altered by AGN are regulated through LXR-RXR heterodimers.

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The expression of T1317-responsive genes was compared to those regulated by WY and AGN. The overlapping genes altered by exposure to the three compounds in wild-type mice are shown in Fig. 2A and the number of genes altered in wild-type mice are shown in Fig. 2B. Remarkably, only one out of 87 overlapping genes was regulated in an opposite manner by WY vs. AGN or WY vs. T1317 (*Armet*, mutated in early stage tumors). The extent of the overlap ranged from ~31-62%. For up-regulated genes T1317 had the greatest overlap with the other two compounds (42%) followed by AGN (34%) and WY (32%). For down-regulated genes AGN had the greatest overlap (62%) followed by T1317 (33%) and WY (31%). Overall, these results indicate that WY, AGN and T1317 regulate an overlapping set of genes in wild-type mice primarily dependent on PPARα or LXRα/LXRβ.

Expression of Fatty Acid Metabolism Genes. The role for PPARα in regulating genes involved in fatty acid metabolism is well known (Hihi et al., 2002). However, there is little information about regulation of these genes by RXR agonists. Genes involved in fatty acid metabolism altered by WY or AGN were grouped into functional categories and compared to those regulated by T1317 (Table 2). Additional genes regulated by WY, AGN and T1317 are found in Supplementary Table 4. The expression of the PPARα gene, *Ppara* was repressed in control PPARα-null mice compared to control wild-type mice (-1.72 fold-change; $p \leq 9.3 \times 10^{-5}$), as expected and expression did not change after compound treatment (data not shown). All of the genes involved in peroxisomal and mitochondrial fatty acid β and ω-oxidation identified here were induced by WY. The genes included three forms of peroxisomal 3-oxoacyl-Coenzyme A thiolase (*Acaa1*) and AW122615, similar to trifunctional protein, beta subunit. The genes also included 1300002P22Rik, 83% similar to human enoyl-Coenzyme A, hydratase/3-hydroxyacyl

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Coenzyme A dehydrogenase (*Ehhadh*) and 4930479F15Rik, known as hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-Coenzyme A thiolase/enoyl-Coenzyme A hydratase (trifunctional protein), beta subunit (*Hadhb*). Induction by WY was abolished in PPARα-null mice (WY, WT vs. WY, Null; Table 2). Eight of the genes including one of the *Acaa1* forms were down-regulated in control PPARα-null mice compared to control wild-type mice (CON, Null, Table 2), consistent with earlier studies describing constitutive activation of β-oxidation genes by PPARα, likely by endogenous activators such as fatty acids (Aoyama et al., 2000).

AGN treatment induced 4 of the β-oxidation genes and both *Cyp4a* genes that were also induced by WY. The AGN-responsive genes included 1300002P22Rik, two forms of *Acaa1*, *Acat2*, *MGC29978, Cyp4a10* and *Cyp4a14*. Regulation of all of these genes except *Cyp4a10* was partially or completely dependent on PPARα as increased expression was lost in PPARα-null mice (AGN, WT vs. AGN, Null, Table 2).

Extra- and intra-cellular fatty acid transport is facilitated by proteins located on cell membranes (e.g., fatty acid transporters) and within the cytoplasm (e.g., fatty acid binding proteins). Three fatty acid binding proteins (*Fabp2; Fabp4; Fabp5*) and a fatty acyl-CoA binding protein (*Dbi*) were induced by WY. Other fatty acid transporters, located on the cell surface or that act as intercellular transporters were either induced (*Abcd3, Slc25a20*) or decreased (*Apom*) by WY. Out of all of these transporters, only *Fabp2* was induced by AGN and in a PPARα-dependent manner. *Slc25a20* was the only transporter dependent on PPARα for basal level expression. These results suggest that WY coordinately induces genes involved in both fatty acid β-oxidation and transport, but that under these conditions, AGN induces only a subset of these genes.

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Genes involved in fatty acid and triglyceride synthesis were regulated by WY or AGN. Fatty acid synthesis genes induced by WY included *Fasn, Helo1-pending* and two forms of *Scd1*. AGN induced *Scd1* and down-regulated *Elov3*. All genes required PPARα for alteration by WY or AGN. The *Fasn* gene was up-regulated by AGN only in PPARα-null mice. *Akr1b7* involved in glycerol lipid synthesis was induced by AGN only. The triglyceride synthesis genes, *Gpam* and *Chk* were induced by WY and AGN, respectively. Induction of *Gpam* may serve as a way to funnel free, potentially toxic fatty acids into storage.

Lipases that govern the release of fatty acids from intra- or extra-cellular stores were up-regulated by WY (*Lpl*, *Mgl1*). The preferential up-regulation of these secreted lipases by WY may indicate an increased use of extracellular fat stores for energy (Auwerx et al., 1996). Consistent with this, *Cte1* and *Pte1* which release fatty acids from acyl-CoA stores were up-regulated by WY or AGN and may be involved in the use of fatty acids for β-oxidation (Hunt et al., 1999). Miscellaneous genes involved in fatty acid metabolism and peroxisome proliferation regulated only by WY included *Aldh3a2, Cyp2c37, Cyp2c40, Pex11a,* and *Pltp*.

LXR agonists have well-known effects on fatty acid metabolism including increases in triglyceride synthesis that lead to increases in circulating triglyceride levels. T1317 exposure led to increases in 5 fatty acid β–oxidation genes, 2 ω-oxidation genes, 2 *Fabp* family members and 4 genes involved in fatty acid and triglyceride synthesis. The number of fatty acid metabolism genes that overlapped was greater for WY and T1317 than for WY and AGN (16 genes vs. 10 genes).

We confirmed the expression of some genes involved in fatty acid metabolism by real time PCR (TaqMan) and by westerns. *Cyp4a14*, *Acox1* and *Lpl* gene expression exhibited the same trends that were observed by transcript profiling, i.e., preferential PPARα-dependent up-

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regulation by WY over AGN (Fig. 3A). Expression of the ACO, MFP-II, thiolase and CYP4A proteins was increased in wild-type mice by WY exposure (Fig. 3B). AGN exposure led to weaker induction of ACO and CYP4A compared to WY. AGN did not appreciably induce MFP-II or thiolase, consistent with the gene array results. Induction of all proteins was dependent on PPARα. These studies illustrate the coordinate regulation of a large number of fatty acid metabolism and transport genes by WY, and to a lesser extent, AGN.

Altered Expression of Cholesterol Synthesis Genes. Nine genes involved in cholesterol synthesis were up-regulated by AGN exposure. Four of these genes were PPARα-dependent. We examined the expression of four cholesterol synthesis genes by TaqMan (Fig. 3C). *Cyp51, Fdft1*, *Fdps*, and *Idi1* exhibited increased expression by AGN in wild-type mice. Although expression of the four genes appeared to be increased by AGN in PPARα-null mice, the increases did not reach significance. In addition, AGN altered expression of bile acid synthesis genes including decreases in *Cyp7b1* and increases in *Ltb4dh*. The AGN-regulated genes involved in cholesterol and bile acid synthesis overlapped with those regulated by T1317. Although the results are consistent with AGN coordinately regulating cholesterol synthesis genes, a role for PPARα needs to be examined further. This is the first study that we are aware of that demonstrates the coordinated regulation of cholesterol synthesis genes by a rexinoid. Further work is required to determine if RXR activation by AGN directly or indirectly regulates these genes.

Coordinate regulation of inflammation genes by WY. WY exposure resulted in coordinate decreases in the expression of genes elevated during times of acute or chronic inflammation including the acute phase response. Acute phase proteins (APP) included *Apcs, Hpsn, Orm1,*

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Orm2 and *Saa2*. Expression of serum amyloid components is also positively associated with increases in very low-density lipoprotein and triglyceride levels (Kindy et al., 2000). Seven components of the complement cascade that are also APP were down-regulated by WY. Decreases in APP expression may be due in part to the decrease in expression of leukemia inhibitory factor receptor (*Lifr*) which dimerizes with gp130 and controls interleukin-6-dependent expression of the APP. Although AGN and T1317 down-regulated *Lifr*, there was no coordinate down-regulation of the same inflammation genes as WY, indicating other genes may be targets of the anti-inflammatory properties of T1317 (Tontonoz and Mangelsdorf, 2003).

PPARα-dependent and -independent Induction of Cell Proliferation in Mouse Liver by AGN. Both PPARα and RXR agonists increase cell proliferation in rat liver (Corton et al., 2000; Standeven et al., 1997). The ability of RXR agonists to increase cell proliferation in mouse liver and dependence on PPARα is not known. Increases in liver to body weights were observed after WY or AGN treatment in wild-type but not PPARα-null mice. (Fig. 4A). We measured heptocellular proliferation after AGN exposure and compared that to induction by WY. Because chemicals can increase cell proliferation differentially across the liver lobule (Bahnemann and Mellert, 1997), we measured hepatocellular proliferation in different lobular zones. In wild-type mice, WY increased hepatocyte proliferation in the centrilobular region only (zone 3) (Fig. 4B). AGN induced hepatocyte proliferation in wild-type mice in zones 2 and 3, being strongest in zone 3. Increases in hepatocyte proliferation by WY were abolished in PPARα-null mice. PPARα-null mice exposed to AGN retained levels of hepatocyte proliferation in zone 2 but exhibited decreases in zone 3 proliferation compared to wild-type mice. Thus, the increases in hepatocyte proliferation by AGN had both PPARα-dependent and -independent

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components. Cell proliferation occurred in the absence of cell death either through cytotoxicity or overt increases in apoptosis (data not shown).

The cell cycle is controlled by the regulated expression and degradation of cyclins, cyclin-dependent kinases (*Cdk*) and *Cdk* inhibitors. The maturation promoting factor (MPF) is a universal regulator of the G_2/M transition (Doree and Hunt, 2002). AGN exposure led to increased expression of MPF components including *Ccnb1, Ccnb2, Cdc2a* as well as *Ccna2* that may interact with MPF (Arooz et al., 2000) (Table 2).

Components of the anaphase promoting complex are required for the degradation of CDK1 and entrance into telophase and cytokinesis. There were AGN-induced increases in the components of APC (*Anapc5*) or proteins that negatively regulate the APC (*Mad2l1*; *Cdc20* and *Kif4*). Negative regulators of the cell cycle also exhibited increased expression including p21 (*Cdkn1a*) and p18 (*Cdkn2c*). Most of the cell cycle, kinetochore, anaphase and DNA structural genes were regulated by AGN through a PPARα-dependent mechanism. Compared to AGN very few cell cycle genes were altered by WY. This may be attributed to the kinetics of WY-induced cell proliferation which peaks around 48 hours after initial exposure (Corton, unpublished observations). The kinetics of AGN induction of cell proliferation in the mouse liver is not known, but the preponderance of AGN-regulated cell cycle genes may reflect delayed cell proliferation compared to WY.

We confirmed the expression of cell cycle genes identified by transcript profiling (Fig. 4C). The genes *Ccnb1, Cdc20, Cdc2a* and *Mad2l1* exhibited similar expression profiles. AGN strongly induced expression in wild-type mice while induction in PPARα-null mice was greatly attenuated but in most cases still significantly altered. *Ccna2* was induced by AGN in wild-type mice but not in PPARα-null mice. Changes in *Cdkn1a* did not reach statistical significance.

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Discrepancies between the transcript profile and TaqMan studies exist (i.e., *Ccnb1*, *Cdc2a*, *Cdkn1a*) highlighting the need to determine more subtle expression results using multiple methods. Collectively, these results identify genes that may be involved in both PPARα-dependent and -independent hepatocyte proliferation induced by AGN.

Miscellaneous genes regulated by WY and AGN. Exposure to WY or AGN resulted in altered expression of additional genes which fell into multiple categories (Supplementary Table 4). The type I deiodinase gene (*Dio1*), down-regulated by AGN in mice (Macchia et al., 2002), was down-regulated by AGN but not WY in both wild-type and PPARα-null mice. Many genes involved in proteolysis were altered by WY including up-regulation of proteasomal components and down-regulation of genes involved in proteolytic cascades. A large number of genes have little or no information regarding their function or their possible role in the phenotypic effects of these compounds.

Discussion

The obligate role for RXR as a heterodimer partner for class II NR is well known, but the genes regulated by rexinoid signaling have not been systematically identified. Here, transcript profiling has been used to generate a comprehensive view of transcriptional alterations in liver genes after exposure to an RXR agonist or a PP in wild-type and PPARα-null mice. The regulated genes fell into a number of categories and their regulation can be explained based on prior knowledge of NR-RXR interactions in the liver (Fig. 5). The Class I and II genes are those activated by WY or WY and AGN and are completely dependent upon PPARα for chemical-

induced responses. A large body of evidence supports a dominant role for the PPARα-RXR heterodimer in PP-regulated gene expression (Corton et al., 2000; Hihi et al., 2002). WY-treated mice harboring a liver-specific deletion of the RXRα gene showed greatly reduced hepatic induction of *Acox1*, *Cyp4a1*, and *Fabp1* (Wan et al., 2000). RXR agonists increased the expression of genes under control of PPARα including *Fabp1* (Poirier et al., 1997), bifunctional enzyme (also known as MFP-I) and *Acox1* (Mukerjee et al., 1998; Standeven et al., 1997), and fatty acid transfer protein and acyl-CoA synthetase (Martin et al., 2000). The RXR agonist LGD1069 increased the expression of Cyp4a, thiolase, *Acox1* as well as cholesterol levels in wild-type but not PPARα-null mice (Ouamrane et al., 2003). Many of the Class I and II genes are likely activated by endogenous activators of PPARα as we identified many of these genes down-regulated in control PPARα-null mice versus control wild-type mice, especially genes involved in fatty acid β-oxidation. PPARα interacts with other NR and co-activators (summarized in Corton et al., 2000) that may alter DNA binding specificity or transcriptional activity. Thus, some of the Class I and II genes could be regulated by mechanisms other than through a PPAR-RXR heterodimer. In particular, there is no evidence that a PPARα-RXR heterodimer is directly involved in down-regulating expression of PP-regulated genes. PPARα negatively regulates genes under control of c-jun or NF-kB through interactions with and sequestration of their subunits (Delerive et al., 1999). This mechanism is consistent with a large number of Class I genes involved in inflammatory responses down-regulated by WY that are known targets of NF-kB.

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The vast majority of genes regulated by WY were PPARα-dependent, an expected finding given the important role PPARα plays in mediating the phenotypic responses of PP (Klaunig et al., 2003). However, a modest number of genes altered by WY in PPARα-null mice were identified and they fell into three groups. The first group, consisted of genes regulated only in PPARα-null mice (class VII) and were dominated by down-regulated genes. Genes in the second group were regulated similarly by WY in both strains. The overall expression changes in this group were relatively weak, possibly due to dilution of differentially-regulated transcripts originating in minor cell types of the liver by hepatocyte transcripts. Genes in the third group were altered in an opposite manner in the two strains, reminiscent of estrogen receptor ligands that demonstrate agonistic or antagonistic properties depending on the cell type in which they are measured (McDonnell et al., 2001). Additional experiments are required to determine if these genes are regulated through complexes of PPARβ or PPARγ with cell-type specific co-activators/co-repressors or through alternate mechanisms.

We unexpectedly identified a large percentage of genes (~80%) that were regulated predominantly by AGN and require PPARα for most, if not all of their changes (Class III genes). The identification of cell cycle genes in this class is consistent with the PPARα-dependent increases in centrilobular hepatocyte proliferation observed in AGN-treated wild-type but not PPARα-null mice. Given the dependence of regulation on PPARα and the specificity of AGN for RXR family members, these genes are most likely regulated through PPARα-RXR heterodimers. Some nuclear receptors within a heterodimer are transcriptionally silent under different promoter-specific contexts (Westin et al., 1998), but this is the first example, to our

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knowledge of the identification of genes that require PPARα for expression but that are not activated by a PPARα ligand to a significant extent. It will be of interest to determine if other rexinoids primarily rely on PPARα for gene expression changes in the liver and whether these genes are nonresponsive to other PP.

RXR is a promiscuous heterodimer partner of many nuclear receptors in the liver, and we identified genes altered by AGN in a PPARα-independent manner (Class IV and VI genes). AGN might regulate these genes either through RXR-RXR homodimers or through heterodimers of RXR and class II NR other than PPARα. Given the significant overlap in the transcript profiles of AGN and T1317, it is possible that many of the Class IV genes are regulated through LXR-RXR heterodimers. Additional evidence for class VI genes comes from studies in which 9-cis retinoic acid or a rexinoid could activate PPARα target genes or responses in PPARα-null mice (Ouamrani et al., 2003; Ijpenberg et al., 2004) and 9-cis retinoic acid could rescue the hypothermic phenotype observed in fasted PPARα-null mice (Ijpenberg et al., 2004).

LXRα/LXRβ and PPARα regulate alternate pathways of fatty acid synthesis and catabolism. Functional antagonism between PPARα and LXR would help ensure that these opposing pathways are not simultaneously activated. Evidence for antagonism between PPARα and LXR comes from in vitro trans-activation studies as well as analysis of a small number of genes in the mouse liver after short-term exposure to WY or T1317 (Yoshikawa et al., 2003; Ide et al., 2003). PPARα activation was shown to inhibit activation of LXR-regulated genes and reciprocally, LXR activation inhibited PPARα-regulated gene expression. Antagonism under these conditions

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likely comes from titration of shared RXR as well as the formation of inactive PPARα-LXR heterodimers (Miyata et al., 1996; Yoshikawa et al., 2003; Ide et al., 2003). If this antagonism existed in the mouse liver, we would predict that activation of PPARα would lead to opposite regulation of LXR-activated genes and vice versa. Under the conditions of our exposures, we found no evidence for antagonism. First, all but one of the 49 genes regulated by both WY and T1317 were regulated in the same direction. Although T1317 suppressed expression of PPARα targets *Hmgcs, Acox1, Cpt1* and *CYP4A2* in the livers of fasted mice after 18 hours (Ide et al., 2004), a 7 day exposure to T1317 had either no effect on these genes or increased expression of *Cyp4a* family members (Stulnig et al., 2002). Secondly, out of the 64 genes constitutively regulated by PPARα in control mice, we found only one that was regulated in a manner that indicated antagonism by T1317, i.e., constitutively up-regulated in control wild-type mice vs. control PPARα-null mice and down-regulation by T1317 in wild-type mice vs. control wild-type mice. Discrepancies between our work and those reported earlier could be partly due to differences in exposure times for T1317 and WY or the use of in vitro systems to determine antagonism in which PPARα and LXR were highly expressed to non-physiological levels. Our work indicates that PPARα and LXR activate an overlapping set of genes involved in both fatty acid catabolism as well as synthesis. The physiological advantage of activating these opposing pathways remains to be determined.

Mechanisms of cross-talk between PPARα and LXR likely exist that do not involve competition for RXR. Increased expression of the LXR gene itself and LXR-regulated genes by PP and PPARα could occur through the peroxisome proliferator response elements found in the

promoters of the mouse (Tobin et al., 2000) and human (Lafitte et al., 2001) LXR genes. LXR gene transcripts are increased in *in vitro* models and in mouse liver after exposure to PP (Tobin et al., 2000). The PPARα agonist clofibrate increases the levels of the endogenous activators of LXR, 25- and 27-hydroxycholesterol in the livers of rats (Guan et al., 2003). Although the basis for LXR regulation of PPARα genes remains elusive, WY may regulate LXR genes through increasing LXR-RXR heterodimer formation and activation. Transcript profiling in PP-treated wild-type and LXR-null mice will help to identify LXR-dependent and –independent genes regulated by PPARα.

In summary, these studies reinforce the dominant role played by PPARα in the regulation of multiple classes of genes by PPAR and RXR agonists in the mouse liver. The significant overlap in the transcript profiles regulated by PPARα, RXR and LXR support the concept of a PPARα-RXR-LXR axis in the liver that acts to control lipid homeostasis.

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Footnotes

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FIGURE LEGENDS

Fig. 1. Genes regulated by a PPAR or RXR agonist in mouse liver. Wild-type (PPARα, +) or PPARα-null (PPARα, -) mice were treated with three consecutive daily doses of either WY-14,643 (WY), AGN194,201 (AGN) or methylcellulose carrier as described in Materials and Methods. Genes whose expression was significantly ($p \leq 0.001$; ≥1.5-fold or ≤-1.5-fold change) different between two or more groups were placed into 9 different categories (I – IX) based on their expression patterns (see text) and then rank-ordered. Red and green lines signify genes that are either up- or down-regulated, respectively after chemical treatment compared to control mice in each strain. The scale represents fold-changes (red, up-regulated; green, down-regulated; black, no change).

Fig. 2. Overlap in WY-, AGN- and T1317-regulated genes. (A) Compound-specific and overlapping genes regulated by WY, AGN and T1317. All genes regulated by WY and AGN in wild-type and PPARα-null mice were compared to the genes regulated by T1317 in wild-type and LXRα/LXRβ mice as described in the text. Genes were grouped into categories based on alteration by one or more compounds in wild-type mice and then sorted based on fold-change. Genes regulated only in PPARα or LXRα/LXRβ mice are not shown. The figure illustrates the overlap in the expression behavior of many of these genes. (B) Overlap in the up- (left) and down- (right) regulated genes by WY, AGN and T1317 in wild-type mice. The Venn diagrams show the number of genes in (A) that are regulated by one or more compounds in wild-type mice.

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Fig. 3. Altered expression of fatty acid and cholesterol metabolism genes by WY and AGN. (A) Altered expression of fatty acid metabolism genes by TaqMan. All groups were normalized to the wild-type controls. Abbreviations: C, control; W, WY; A, AGN. (B) Altered expression of fatty acid metabolizing enzymes. Protein expression was determined by Western blot analysis using polyclonal antibodies against the indicated proteins. Protein levels are expressed as relative intensity values. (C) Altered expression of cholesterol synthesis genes by TaqMan. Error bars are standard errors of the means. *, Significantly different from control wild-type group ($p \leq 0.05$). #, Indicates significant difference from the treated wild-type group ($p \leq 0.05$).

Fig. 4. PPARα-dependent and independent induction of hepatocyte proliferation and modulation of cell cycle gene expression after WY and AGN exposure. Changes in (A) liver to body weights and (B) hepatocyte proliferation. Labeling indices were determined for each of the three lobular zones in the livers of the indicated groups. (C) Confirmation of altered gene expression. Expression of the indicated genes was determined by TaqMan. Abbreviations are the same as in Fig. 2A. Error bars are standard errors of the means. *, Indicates significant difference from the control group ($p \leq 0.05$). #, Indicates significant difference from the treated wild-type group ($p \leq 0.05$).

Fig. 5. Gene classification by nuclear receptor heterodimer targets of WY and AGN. Genes regulated by WY or AGN were classified into major groups based on expression behavior. Classes I–III represent genes that are dependent on PPARα for altered expression after exposure to either WY (Classes I and II) or AGN (Classes II and III). Class IV gene expression after AGN exposure is independent of PPARα expression and could be the result of AGN activation of

RXR dimerized with Class II nuclear receptors (represented by a "?"). Class V genes are AGN-responsive only in the absence of PPARα, i.e., altered regulation is observed in PPARα-null mice but not in wild-type mice and may be due to alleviation of negative regulation by PPARα. Additional classes of WY- and AGN-regulated genes exist and are discussed in greater detail in the text.

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TABLE 1

Comparison between transcript profiles altered by WY, AGN and T1317 using Spearman rank correlation

Chemical, Genotype	WY PPARα-null	AGN WT	AGN PPARα-null	T1317 WT	T1317 LXRα/LXRβ-null
WY, WT	**-1.00**[1] (≤0.0001)[2] n = 5	**0.877** (≤0.0001) n = 41	0.297 (0.303) n = 14	**0.864** (≤0.0001) n = 50	0.000 (>0.99) n = 4
WY, PPARα-null		NA[3]	**0.994** (≤0.0001) n = 8	NA	NA
AGN, WT			**0.858** (≤0.0001) n = 27	**0.882** (≤0.0001) n = 39	- 0.300 (0.624) n = 5
AGN, PPARα-null				**0.835** (≤0.0001) n = 13	0.500 (0.667) n = 3
T1317, WT					0.476 (0.233) n = 8

[1] Spearman rank correlation coefficients

[2] p values. Values in bold indicate a significant correlation ($p \leq 0.05$).

[3]NA, cannot be calculated because of insufficient sample size

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TABLE 2
Gene expression altered by agonists of PPAR, RXR and LXR in mouse liver

Gene Name[a]	Gene Description[a]	Accession #[a]	Class[b]	WY, WT[c]	WY, Null	AGN, WT
Fatty Acid Catabolism - β-Oxidation						
1300002P22Rik	RIKEN cDNA 1300002P22 gene	AJ011864	6	16.29[d]		6.36
4930479F15Rik	RIKEN cDNA 4930479F15 gene	AW122615	1	2.11		
Acaa1	acetyl-Coenzyme A acyltransferase 1	AI530403	2	10.24		4.13
Acaa1	acetyl-Coenzyme A acyltransferase 1	AI841705	2	3.27		1.55
Acadl	acetyl-Coenzyme A dehydrogenase, long-chain	U21489	1	2.43		
Acadm	acetyl-Coenzyme A dehydrogenase, medium chain	U07159	1	2.33		
Acat2	acetyl-Coenzyme A acetyltransferase 2	M35797	2	1.52		2.34
Acox1	acyl-Coenzyme A oxidase 1, palmitoyl	AF006688	1	2.26		
Dci	dodecenoyl-Coenzyme A delta isomerase	Z14050	1	3.49		
Decr1	2,4-dienoyl CoA reductase 1, mitochondrial	AI844846	1	3.09		
Ech1	enoyl coenzyme A hydratase 1, peroxisomal	AF030343	1	2.75		
Facl5	fatty acid Coenzyme A ligase, long chain 5	AI838021	1	1.53		
Hadh2	hydroxyacyl-Coenzyme A dehydrogenase type II	U96116	1	2.62		
Hsd17b4	hydroxysteroid (17-beta) dehydrogenase 4	X89998	1	1.84		
MGC29978	3-ketoacyl-CoA thiolase B	AW012588	2	3.7		2.2
Peci	peroxisomal delta3, delta2-enoyl-Coenzyme A isomerase	AI840013	1	2.12		
Fatty Acid Catabolism - ω-Oxidation						
Cyp4a10	cytochrome P450, family 4, subfamily a, polypeptide 10	AB018421	6	5.07		3.48
Cyp4a14	cytochrome P450, family 4, subfamily a, polypeptide 14	Y11638	2	5.47		4.05
Fatty Acid Transport						
Abcd3	ATP-binding cassette, sub-family D (ALD), member 3	L28836	1	1.94		
Apom	apolipoprotein M	AA655303	1	-1.52		
Dbi	diazepam binding inhibitor	X61431	1	1.78		
Fabp2	fatty acid binding protein 2, intestinal	M65034	2	2.48		1.94
Fabp4	fatty acid binding protein 4, adipocyte	M20497	1	6.89		
Fabp5	fatty acid binding protein 5, epidermal	AJ223066	1	3.88		
Slc25a20	solute carrier family 25 (mitochondrial carnitine/acylcarnitine translocase), member 20	AB017112	1	1.78		
Fatty Acid/Triglyceride Synthesis						
Akr1b7	aldo-keto reductase family 1, member B7	J05663	3			6.24
Chk	choline kinase	AB030621	3			1.61
Elovl3	elongation of very long chain fatty acids-like 3	U97107	3			-2.5
Fasn	fatty acid synthase	X13135	9	2.05		
Gpam	glycerol-3-phosphate acyltransferase, mitochondrial	U11680	1	1.53		
Helo1-pending	homolog long chain polyunsaturated fatty acid elongation enzyme	AI852098	1	1.58		
Scd1	stearoyl-Coenzyme A desaturase 1	M21285	2	1.52		1.58
Scd1	stearoyl-Coenzyme A desaturase 1	M21285	2	1.82		1.93
Lipase						
Lpl	lipoprotein lipase	AA726364	1	7.98		
Lpl	lipoprotein lipase	M63335	1	6.79		

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Mgll	monoglyceride lipase	AI846600	1	2.47		
Miscellaneous – Fatty Acid Metabolism						
Cte1	cytosolic acyl-CoA thioesterase 1	Y14004	2	11.08		2.87
Cyp2c37	cytochrome P450, family 2. subfamily c, polypeptide 37	AF047542	2	-2.5		-1.64
Cyp2c40	cytochrome P450, family 2, subfamily c, polypeptide 40	AF047727	1	-1.73		
Pex11a	peroxisomal biogenesis factor 11a	AF093669	1	2.26		
Pltp	phospholipid transfer protein	U28960	1	2.77		
Pte1	peroxisomal acyl-CoA thioesterase 1	AW046123	1	2.5		
Srebf1	sterol regulatory element binding factor 1	AI843895	7		-1.7	
Cholesterol Synthesis						
Cyp51	cytochrome P450, 51	AW122260	3			2.33
Dhcr7	7-dehydrocholesterol reductase	AF057368	3			1.85
Fdft1	farnesyl diphosphate farnesyl transferase 1	D29016	4			2.28
Fdps	farnesyl diphosphate synthetase	AI846851	4			1.82
Fdps	farnesyl diphosphate synthetase	AW045533	4			1.9
Hmgcs1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	AW124932	4			3.22
Idi1	isopentenyl-diphosphate delta isomerase	AA716963	4			2.32
Lss	lanosterol synthase	AW060927	3			2.65
Nsdhl	NAD(P) dependent steroid dehydrogenase-like	AL021127	3			1.95
Nsdhl	NAD(P) dependent steroid dehydrogenase-like	AW106745	3			1.73
Nsdhl	NAD(P) dependent steroid dehydrogenase-like	AW106745	3			1.87
Sc4mol	sterol-C4-methyl oxidase-like	AI848668	4			1.82
Bile Acid Synthesis						
Aldh3a2	aldehyde dehydrogenase family 3, subfamily A2	U14390	1	3.99		
Aldh3a2	aldehyde dehydrogenase family 3, subfamily A2	AV276715	1	3.59		
Cyp7a1	cytochrome P450, family 7, subfamily a, polypeptide 1	L23754	5			
Cyp7b1	cytochrome P450, family 7, subfamily b, polypeptide 1	AV141027	3			-2.13
Cyp7b1	cytochrome P450, family 7, subfamily b, polypeptide 1	U36993	2	-1.52		-2.23
Ltb4dh	leukotriene B4 12-hydroxydehydrogenase	AA596710	3			2.56
Cholesterol- Miscellaneous						
Apoa4	apolipoprotein A-IV	M64248	1	-3.58		
Ldlr	low density lipoprotein receptor	Z19521	5			
Inflammation						
Acute Phase Response						
Apcs	serum amyloid P-component	M23552	1	-2.18		
Hpxn	hemopexin	AV105307	1	-1.73		
Orm1	orosomucoid 1	M27008	1	-1.73		
Orm1	orosomucoid 1	M27008	1	-1.57		
Orm2	orosomucoid 2	M12566	1	-3.85	2.1	
Saa2	serum amyloid A 2	M13521	9	-14.29	3.45	
Saa2	serum amyloid A 2	U60438	9	-25	4.5	
Complement Cascade						
C1qb	complement component 1, q subcomponent, beta polypeptide	M22531	5			
C1qg	complement component 1, q subcomponent, gamma polypeptide	X66295	4			-1.93
C9	complement component 9	X05475	1	-1.73		
Cfh	complement component factor h	M12660	1	-1.93		

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Cfh	complement component factor h	M29009	1	-2		
Cfh	complement component factor h	M29010	1	-1.82		
Cfi	complement component factor i	U47810	1	-1.62		
Hc	hemolytic complement	M35525	1	-1.73		
Mbl2	mannose binding lectin, serum (C)	U09016	1	-1.52		
Histocompatibility						
H2-Aa	histocompatibility 2, class II antigen A, alpha	X52643	9	-20		
H2-T10	histocompatibility 2, T region locus 10	M35244	1	-1.64		
H2-T23	histocompatibility 2, T region locus 23	Y00629	1	-1.67		
Miscellaneous						
Cxcl12	chemokine (C-X-C motif) ligand 12	L12029	1	-1.7		
Fgls-pending	fragilis	AW125390	1	-3.23		
Ii	Ia-associated invariant chain	X00496	5			
Lect2	leukocyte cell-derived chemotaxin 2	AB009687	6	-1.57		-1.89
Lifr	leukemia inhibitory factor receptor	D17444	2	-2.5		-2.71
Lifr	leukemia inhibitory factor receptor	D17444	2	-2.28		-2.44
Ltbr	lymphotoxin B receptor	L38423	8		1.91	
Cell Cycle/Cell Proliferation						
Ccna2	cyclin A2	X75483	3			2.16
Ccnb1	cyclin B1	X64713	3			5.02
Ccnb2	cyclin B2	X66032	4			2.84
Cdc20	cell division cycle 20 homolog (S. cerevisiae)	AW061324	4			5.17
Cdc2a	cell division cycle 2 homolog A (S. pombe)	M38724	3			22.04
Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	AW048937	3			2.23
Cdkn2c	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	U19596	3			1.78
Chaf1b	chromatin assembly factor 1, subunit B (p60)	AI173038	1	4.71		
Nek2	NIMA (never in mitosis gene a)-related expressed kinase 2	AF013166	5			
Plk	polo-like kinase homolog (Drosophila)	U01063	3			2.62
Stk6	serine/threonine kinase 6	U80932	3			4.26
Kinetochore						
Angptl4	angiopoietin-like 4	AI326963	1	-1.86		
Kif20a	kinesin family member 20A	Y09632	3			4.54
Kif2c	kinesin family member 2C	AA007891	3			2.2
Kif4	kinesin family member 4	D12646	3			3.77
Krt2-8	keratin complex 2, basic, gene 8	X15662	1	1.62		
Lamb3	laminin, beta 3	U43298	1	15.63		
Mad2l1	MAD2 (mitotic arrest deficient, homolog)-like 1 (yeast)	U83902	3			5.57
Prc1	protein regulator of cytokinesis 1	AA856349	3			5.28
Smc2l1	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)	U42385	3			2.91
Smc4l1	SMC4 structural maintenance of chromosomes 4-like 1 (yeast)	AA032310	3			3.71
Stim1	stromal interaction molecule 1	U47323	3			1.75
Stmn1	stathmin 1	AI838080	4			23.87
Sycp3	synaptonemal complex protein 3	AW212131	1	2.37		
Anaphase						
Anapc5	anaphase-promoting complex subunit 5	AI847314	3			1.56
Dnclc1	dynein, cytoplasmic, light chain 1	AF020185	1	1.5		

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Mki67	antigen identified by monoclonal antibody Ki 67	X82786	3		4.85
Top2a	topoisomerase (DNA) II alpha	U01915	3		3.89
DNA Structural					
H2afx	H2A histone family, member X	Z35401	3		1.86
H2afz	H2A histone family, member Z	U70494	3		1.98
Hist1h1c	histone 1, H1c	J03482	2	-1.79	-1.86
Hmgn1	high mobility group nucleosomal binding domain 1	X53476	1	1.57	
Hmgn2	high mobility group nucleosomal binding domain 2	X12944	3		1.72
DNA Repair					
Rad51l1	RAD51-like 1 (S. cerevisiae)	U92068	1	1.58	

[a]Gene name, gene description, and accession # are from Affymetrix (Aug. 1, 2003)

[b]Refers to type of regulation by WY and AGN in wild-type and PPARα-null mice. See text for details.

[c]Treatment groups: WY, WT – treatment with WY for 3 days in wild-type mice normalized to control treated wild-type mice. WY, Null – treatment with WY for 3 days in PPARα-null mice normalized to control treated PPARα-null mice. AGN, WT – treatment with AGN194204 for 3 days in wild-type mice normalized to control treated wild-type mice. AGN, Null – treatment with AGN194204 for 3 days in PPARα-null mice normalized to control treated PPARα-null mice. T1317, WT – treatment with T0901317 for 7 days in wild-type mice normalized to control treated wild-type mice. T1317, Null – treatment with T0901317 for 7 days in LXRα/LXRβ-null mice normalized to control treated LXRα/LXRβ-null mice. CON, Null – control treated PPARα-null mice normalized to control treated wild-type mice.

[d]Numbers are in fold-changes

[e]Only those gene changes altered by T1317 and that overlap with one of the WY or AGN treatment groups are shown. Additional T1317-regulated genes are described in Stulnig et al. (2002).

Figure 1

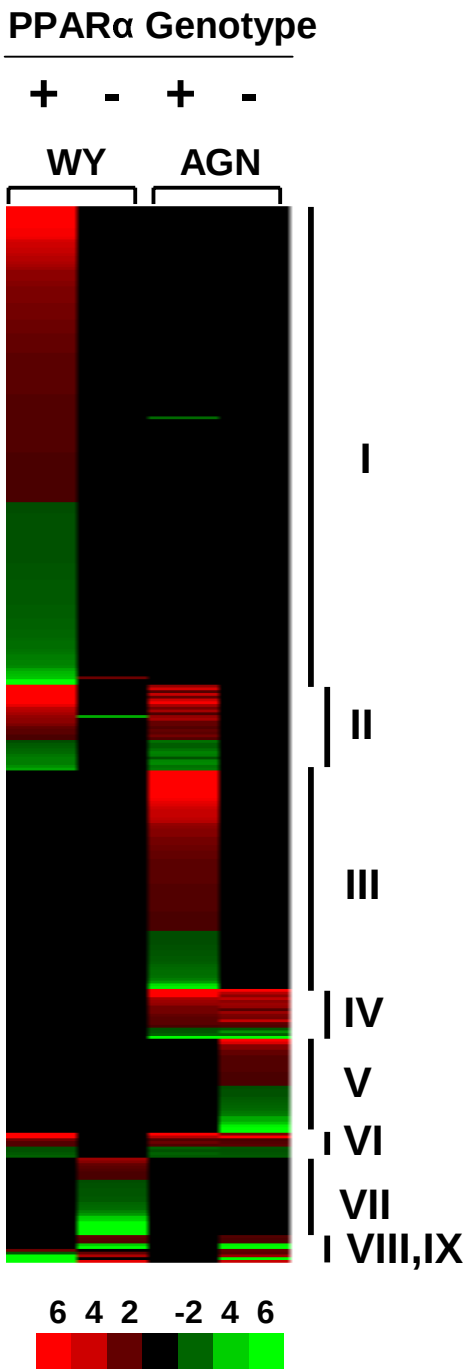

Figure 2A

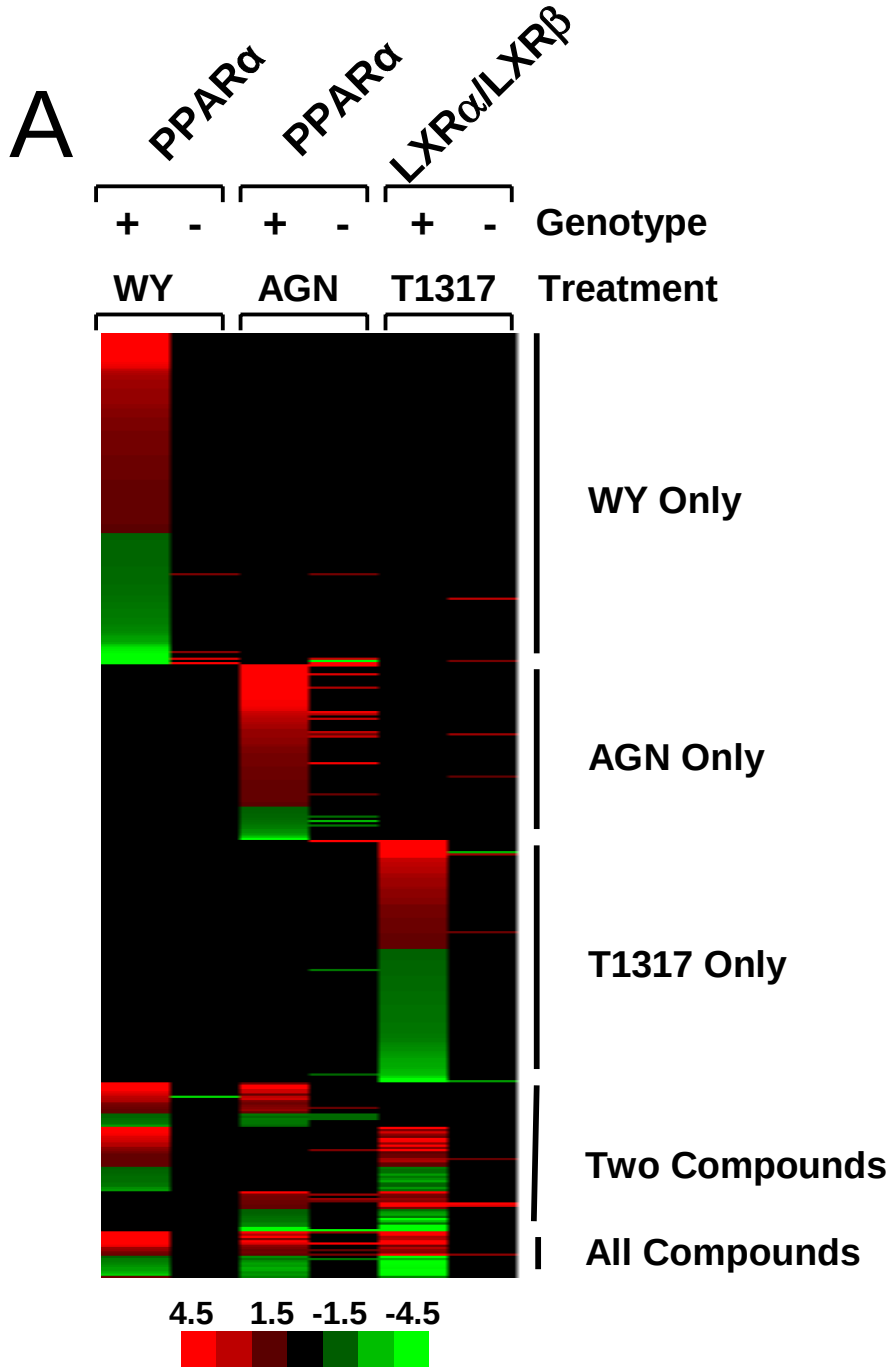

Figure 2B

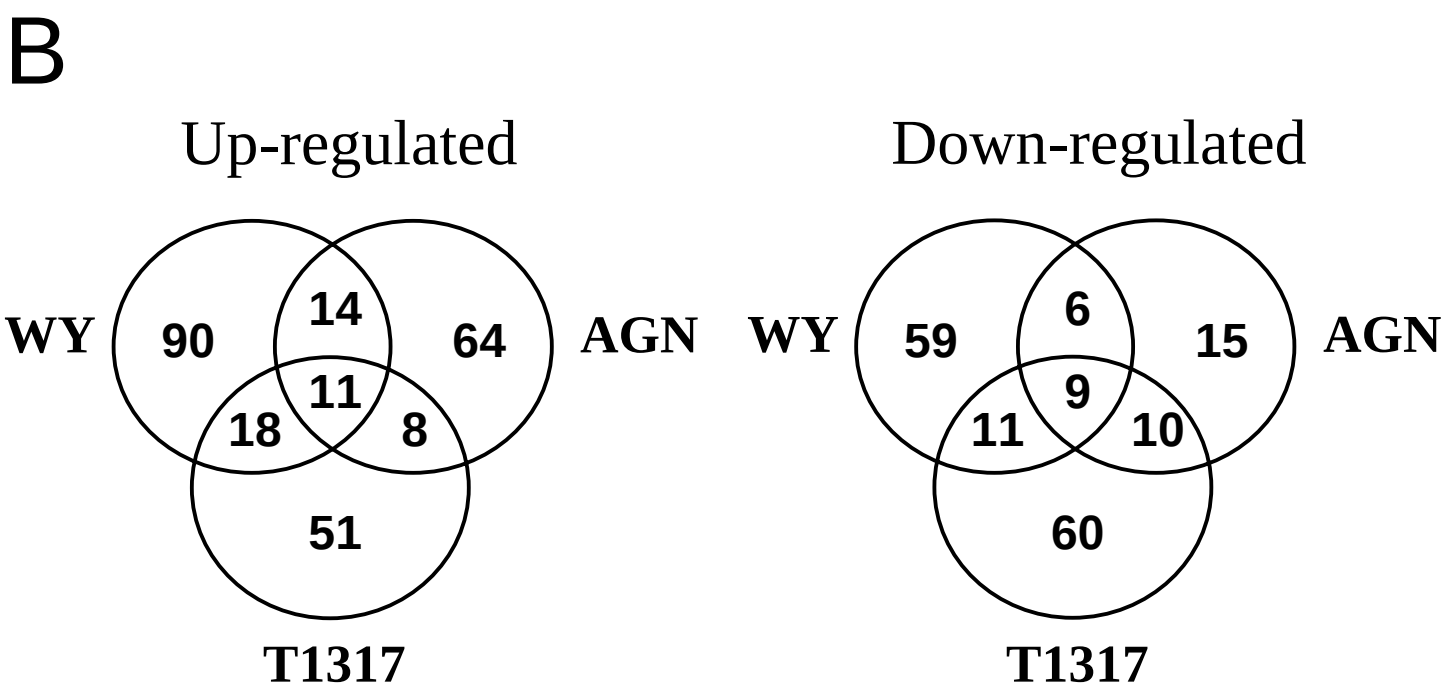

Figure 3A

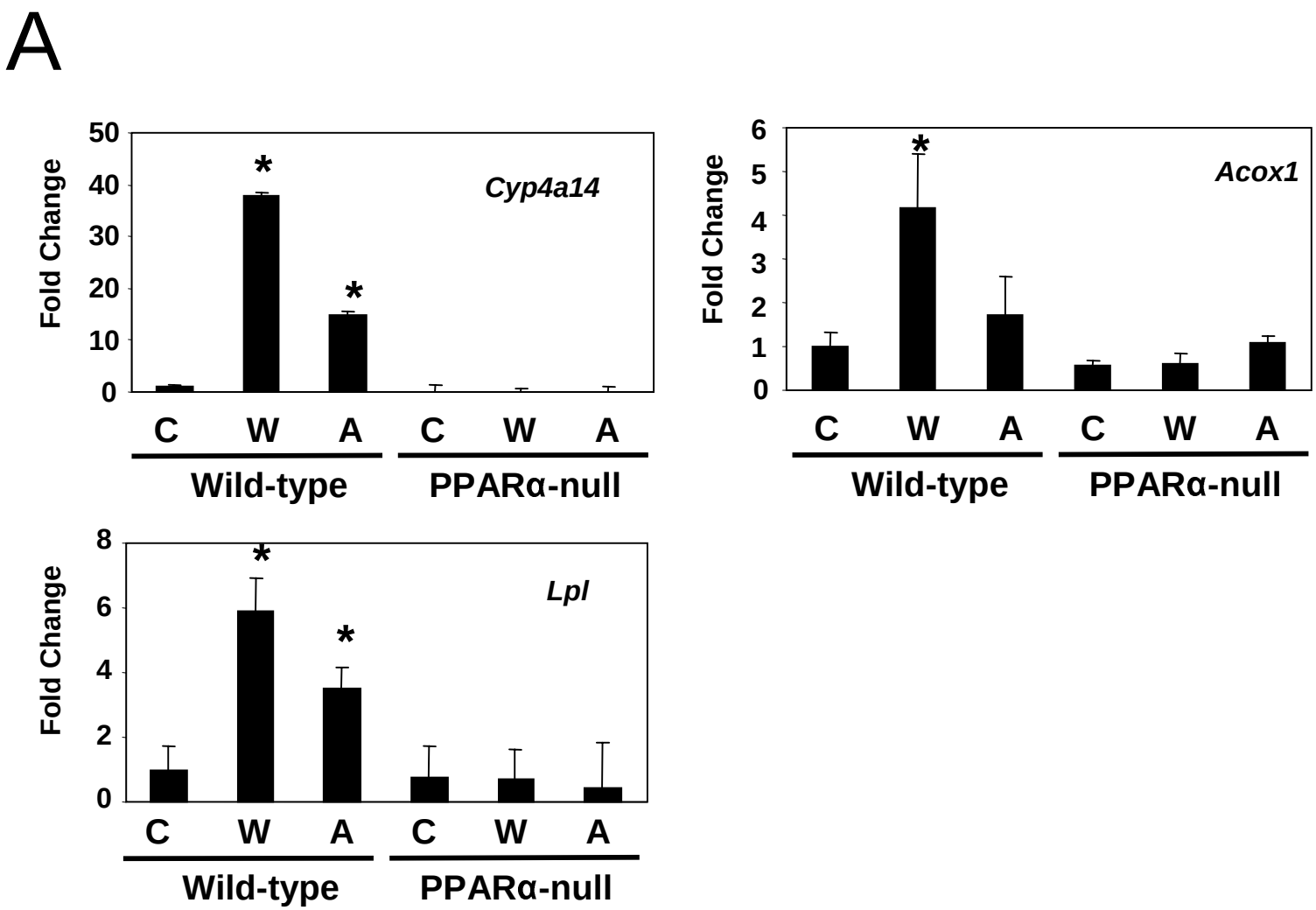

Figure 3B

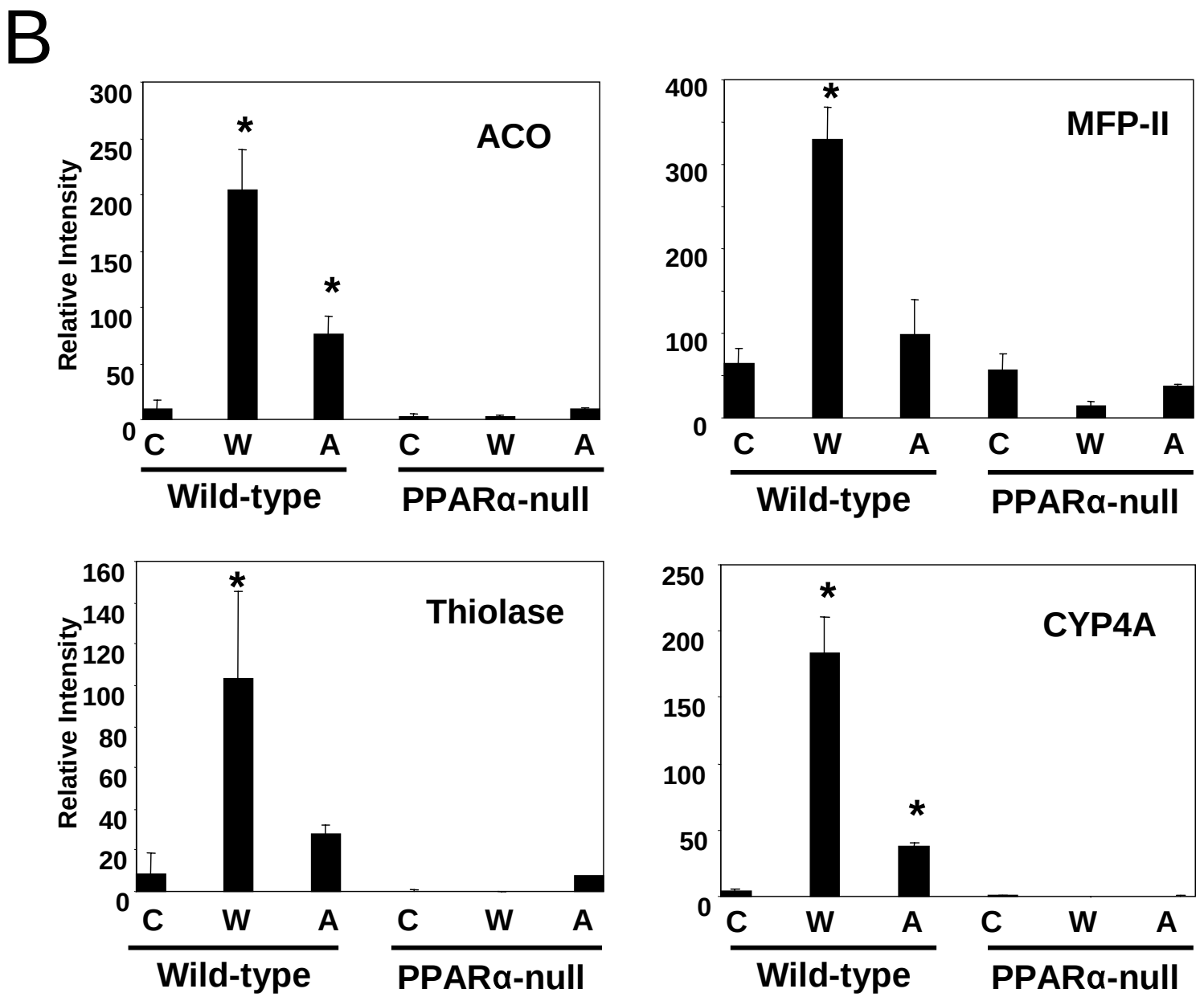

Figure 3C

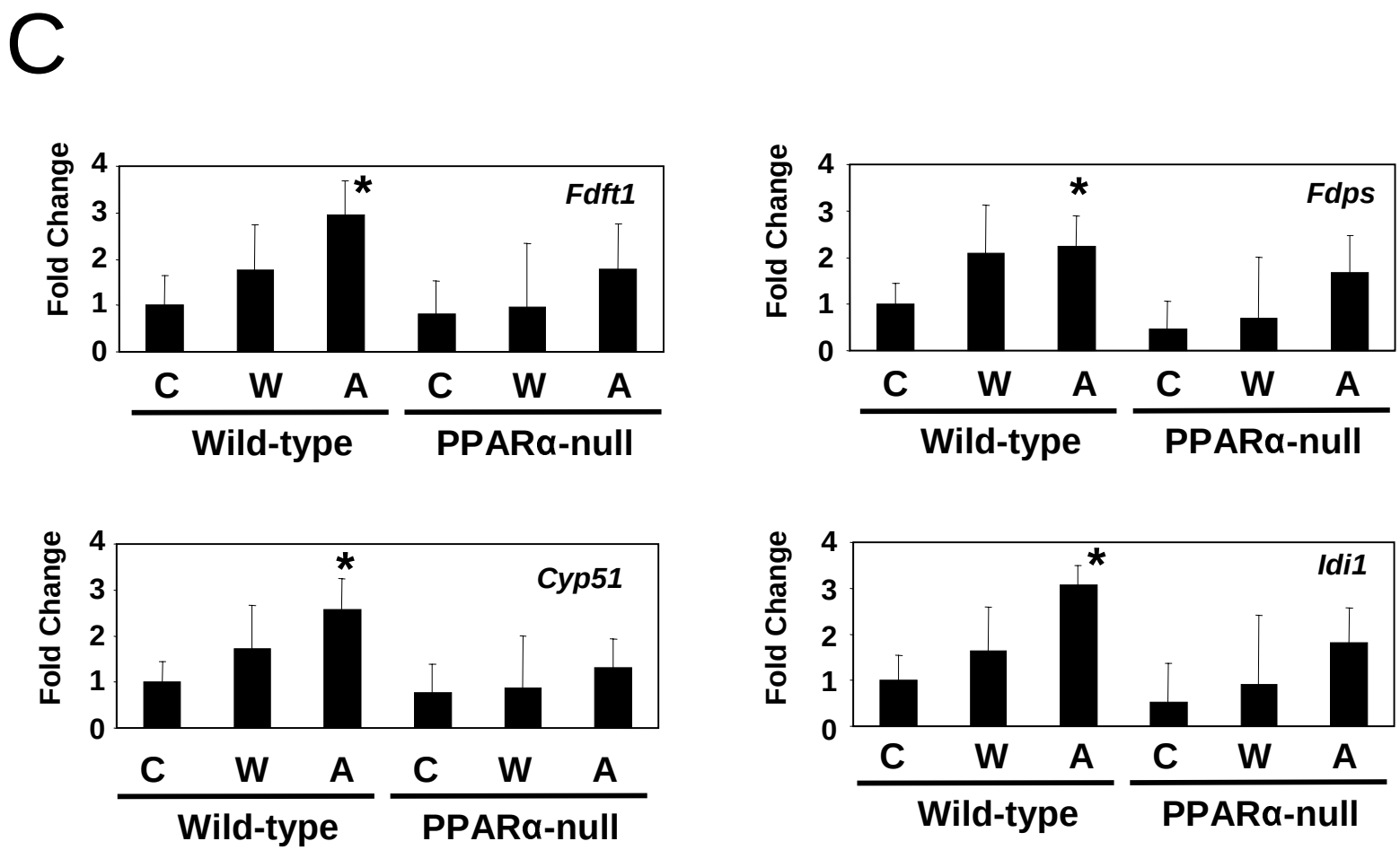

Figure 4A

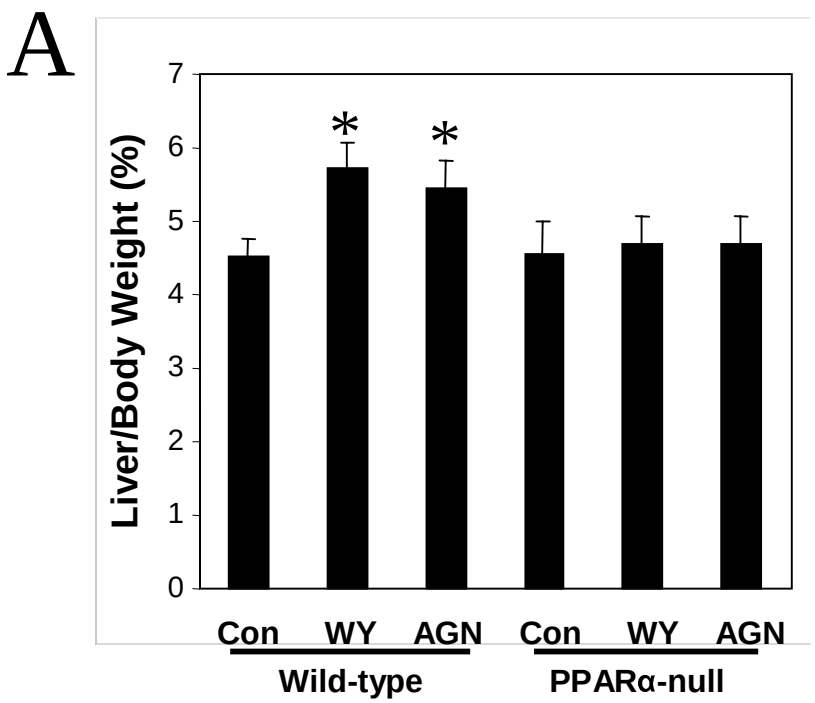

Figure 4B

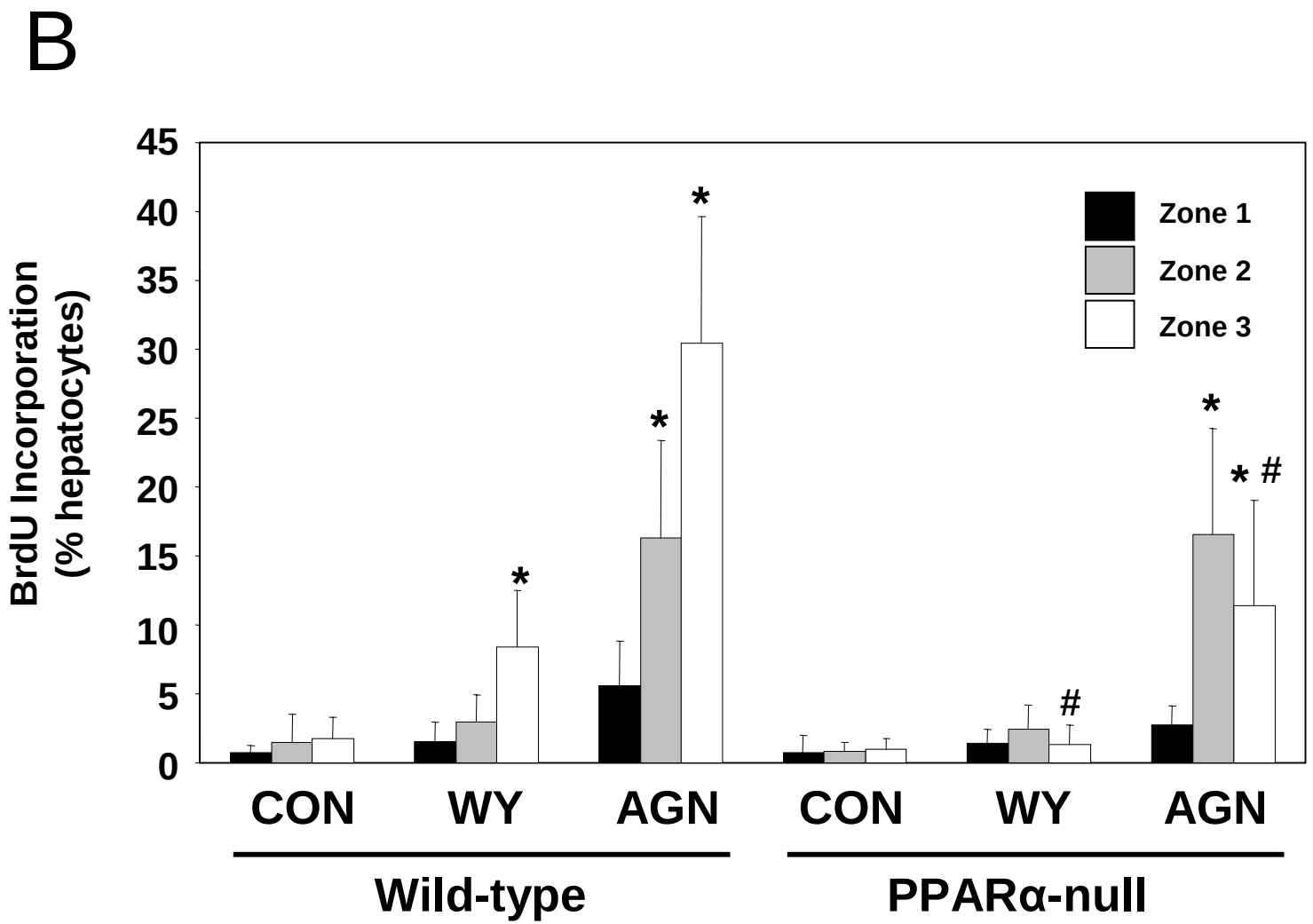

Figure 4C

C

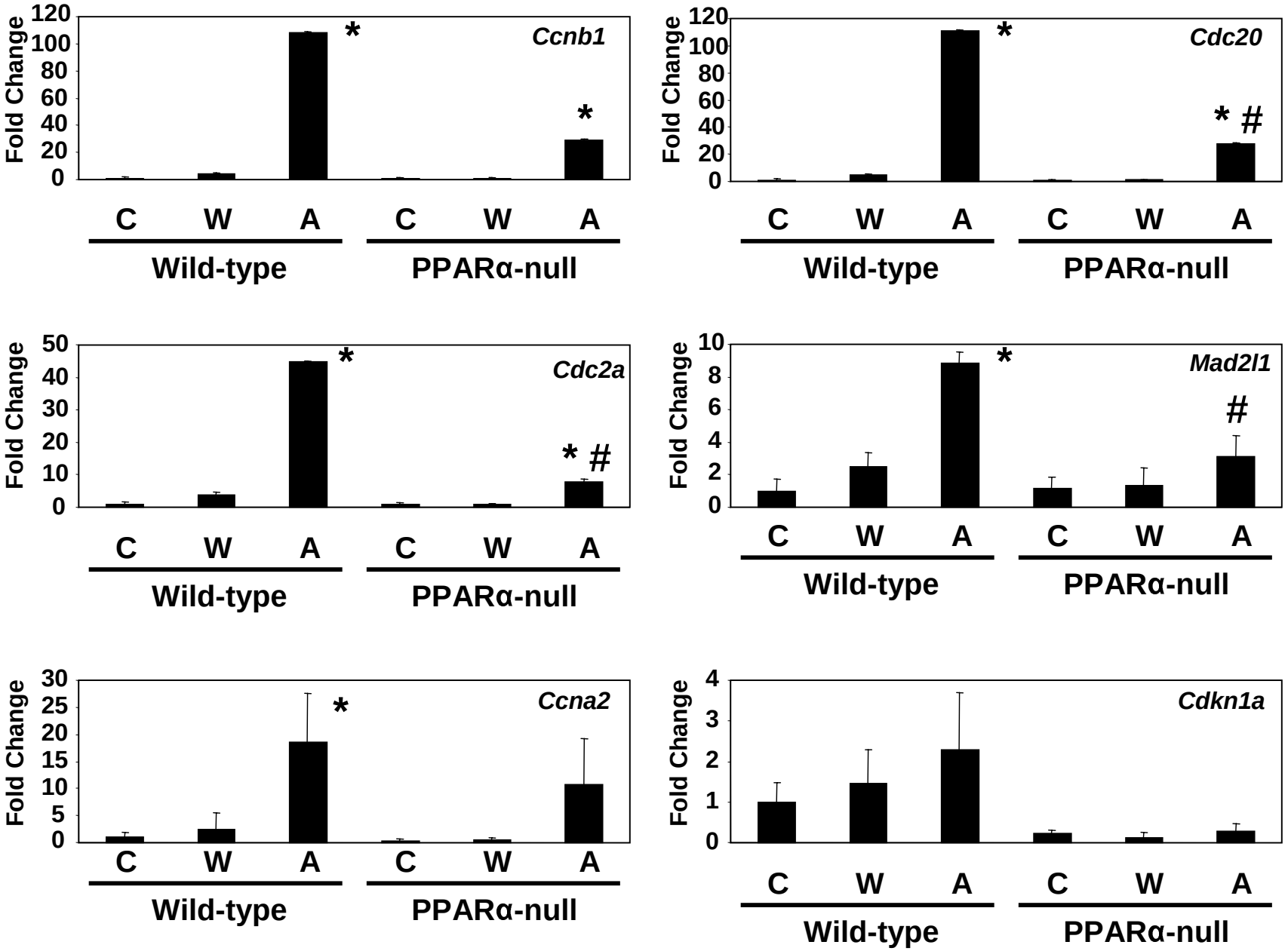

Figure 5