Introduction
To investigate PXR- and CAR-regulated endogenous and species specific functions we performed transcript profiling and pathways analysis on RNA extracted from the livers of mice in which the murine pregnane X receptor (PXR) and constitutive androstane receptor (CAR) genes were removed (PXR KO, CAR KO mice) and/or replaced with human PXR or CAR (hPXR, hCAR mice). Liver RNA expression profiles of these mouse lines, relative to C57BL/6j wild type (WT) mouse liver, were assessed using Agilent whole mouse genome expression microarrays. Rosetta Resolver™ software was used to generate a ‘signature’ list of significantly (p<0.01) altered genes for each mouse line. Ingenuity Pathways Analysis™(IPA) software, was used to examine differences between the knock out (KO) and humanised lines and to verify regulation by these receptors. Effects of the PXR/CAR KO’s and humanisations in classical and functional pathways were assessed by examining the polarity of RNA expression changes to genes contained in pathways that were over-represented in the signature lists relative to their representation in those pathways in the IPA database.

Questions?
• How does the polarity of gene expression alterations, relative to WT, in PXRKO vs hPXR and CARKO vs hCAR mouse liver compare in pathways commonly affected in these models?
• What genes/pathways are uniquely altered in PXRKO and hPXR liver and CARKO or hCAR liver?
• Does investigation of gene/pathway alterations in PXRKO, CARKO or hPXR, hCAR mouse liver, relative to WT mouse liver, offer insight for understanding endogenous roles for these receptors?
• What is the toxicological relevance of gene/pathway alterations in the liver of these KO and transgenic mouse models?

Experimental outline
• RNA isolated from WT, PXRKO, hPXR, CARKO and hCAR mouse liver and labeled with either cyanine 3 (Cy3) or cyanine 5 (Cy5) nucleotides using an Agilent low input RNA labeling kit.
• Test RNA (PXRKO, hPXR, CARKO and hCAR) mouse liver and labeled with either cyanine 3 (Cy3) or cyanine 5 (Cy5) nucleotides using an Agilent low input RNA labeling kit.
• Rosetta Resolver™ software used to identify a signature list of genes significantly (p<0.01) altered, relative to WT, against a set of experimentally derived ‘housekeeping’ genes.
• Signature lists examined using IPA software.
• Venn diagram analysis used to focus IPA analysis on gene/pathway alterations common or unique to the signature lists for each KO and humanised model.
• Representation of genes in these lists assessed in IPA using a one-tailed Fishers exact test.
• Genes from significantly over-represented functional categories loaded into pathway diagrams and overlaid with fold change values to assess the polarity and biological implications of the changes.

Results - PXRKO and hPXR
• Functional categories of lipid and xenobiotic metabolism were overrepresented in a list of 1550 genes that were commonly altered in PXRKO and hPXR mouse liver.
• Genes in these categories were mostly upregulated in PXRKO and down-regulated in hPXR, Figure 1, Table 1.
• There was significant overrepresentation of genes, including several cyp isoforms, in the canonical (classical) pathways of xenobiotic metabolism signalling in the list of genes that were uniquely altered in PXRKO mice.
• Several genes that are upstream regulators and transcriptional targets of nuclear factor erythroid derived like-2 (NF2), a transcription factor involved in regulating the cellular response to oxidative and endoplasmic reticulum stress were altered uniquely (mostly up-regulated) in PXRKO mouse liver. These effects did not occur in hPXR mouse liver.

Results - CARKO and hCAR
• Fatty acid and xenobiotic metabolism pathways were also overrepresented in a list of 2068 genes that were commonly altered in CARKO and hCAR mouse liver.
• Genes in these pathways were altered with similar polarity but with less severity in hCAR mice compared to CARKO mice, Table 2.
• There was overrepresentation of genes in xenobiotic metabolism signalling pathways in the list of genes uniquely altered in CARKO mice.
• Similar pathways were also overrepresented in the hCAR unique signature list albeit with different genes involved.

Summary and Conclusions
• The majority of genes commonly altered in fatty acid/xenobiotic metabolism pathways in PXRKO and hPXR mouse liver were up-regulated in PXRKO mice but the change was reversed in hPXR mice.
• This indicates that hPXR may act endogenously as a repressor of genes in these pathways.
• Induction of several genes regulated by Nrf2 occurred uniquely in PXRKO mouse liver.
• Lack of induction of cytochrome P450 isoforms in hPXR mouse liver was reflected by a lack of alteration to Nrf2 signalling pathways involved in redox homeostasis.
• The majority of expression changes (relative to WT) to genes in liver of NCAR mice in toxicologically relevant pathways were altered in a similar fashion but with less severity than in CARKO mice. This reflects functional replacement of the mouse CAR gene with the human homologue.

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