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Comparative Genomics

Comparative Genomics of Drug Metabolism: Porcine-Human PXR Gene Comparison

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http://www.swinegenomics.com

Abstract

The pregnane X receptor (PXR) plays a crucial role in drug metabolism. It acts as the major transcriptional regulator of cytochrome P-450 monooxygenase 3A4, which metabolizes 60% of all drugs. Recent pharmacodynamic studies have shown that the mouse is an inappropriate model for human clinical drug studies. Therefore, we characterized the swine PXR gene as an alternate model. Gene-specific BACs were isolated from the porcine CHORI 242 library using an in silicone cloning strategy, and primers were designed to identify PXR exons 2 to 9. Amplified BAC-derived products were then sequenced. The genomic sequence from exon 2 to 9 was determined using sequential primer analysis and cloning. A complete swine mRNA sequence was obtained using 3' and 5' RACE with extracted liver and brain mRNA. The swine protein showed 83% identity and 87% similarity with the human PXR protein. Like the human, the swine PXR gene contained a number of predicted splicing variants. Thus, swine liver and brain tissues were then analyzed and two unique splicing variants were detected. One spliced out exon 6 and the second variant completely excised both Exon 6 and 7. Northern blot analysis indicated that PXR was expressed in tissues including the liver, small intestine, heart, kidney, and colon. In addition, a group of pigs representing eight breeds were analyzed for SNPs to permit comparison between human and porcine variants. This characterization of the swine PXR gene will contribute to the development of a swine metabolic medical model.

Introduction

• PXR is a nuclear hormone receptor that transcriptionally regulates cytochrome P4503A4 (CYP3A4). It has been reported that the CYP3A4 is responsible for the catabolism of over 60% of all pharmaceutical drugs currently in use. As PXR plays such a large role in the regulation of CYP3A4, examining this gene is important in the understanding of individual response towards drugs. Consequently, many studies have focused on the characterization of human PXR.

• The human PXR has already been characterized on many levels. Full genomic and mRNA sequences exist, and the peptide structure has been analyzed chemically. PXR has been examined for splicing variants, single nucleotide polymorphisms, tissue expression, and ligand sensitivity. As a more complete description of the human PXR is formed, the important role this gene plays in the metabolism of drugsis better understood.

• It is important to determine a suitable animal model for pharmaceutical drug studies, as it is often unethical to use human participants. While the mouse is a common choice in the development of animal models, it has recently been discovered that it is inappropriate for use in drug studies. Four amino acids cause a difference in PXR activity between mouse and human, and pigs have these 4 amino acids in common with human. This suggests that the swine could act as a more suitable pharmacological model.

Objectives

• Generate genomic and mRNA porcine sequence information to compare to human PXR

• Analyze PXR at the individual level through splice variants and tissue expression

• Examine single nucleotide polymorphisms to characterize PXR within a larger population

Methods and Results

Sequencing

• BACs containing the PXR region were isolated from the CHORI- 242 BAC library.
  – BAC CH242 35F2 was selected for sequencing

• Sequencing was completed through overlapping primers and vector cloning. Completing the mRNA sequence also used 3' and 5' RACE
  – Spanning approximately 10 kb, a complete genomic sequence was determined from exon 2 to exon 9
  – The complete mRNA spans approximately 2.5 kb

• The open reading frame of the swine PXR spans exon 2-9, producing a 421 amino acid long peptide
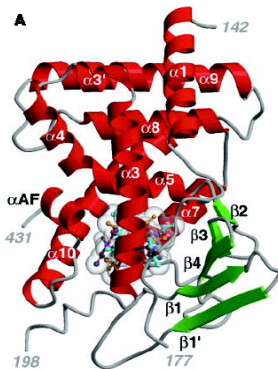

| Table 1. A Comparison of Human and Animal PXR Peptide		
Animal	% Identity	% Similarity
Mouse	77	86
Dog	80	87
Pig	85	91

Splice Variants

• PXR exons 2-9 were amplified from liver, small intestine, brain, and lymph tissues

• Liver and small intestine expressed transcripts of multiple sizes

• Liver and Small Intestine fragments were cloned and sequenced

• Two novel porcine variants were discovered: ssPXR.1 and ssPXR.2

PXR.1
Located in small intestine
Lacks exon 6
42 amino acid deletion

PXR.2
Located in liver
Excises exons 6 and 7
Frame shift- divergent exon 8 and 9 translation
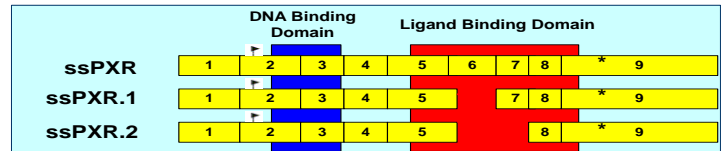
Tissue Expression

A tissue expression profile was established using Northern blot. Eight tissues were analyzed: the heart, kidney, colon, brain, adrenal, and stomach. Of these tissues, the liver and small intestine displayed the greatest level of expression. The heart, kidney, and colon also displayed a minimal amount of expression. The brain, adrenal, and stomach showed none. Two bands were present of approximately 3.2 and 2.5 Kb.
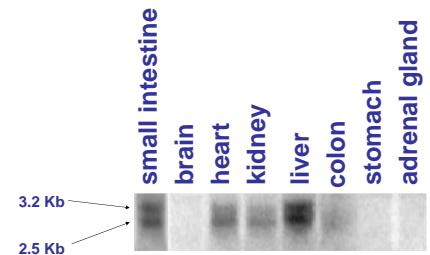
Single Nucleotide Polymorphisms

The exonic regions of PXR were sequenced from a group of pigs that included eight different breeds: Hanford, Sinclair, Yucatan, Duroc, Pietrain, Hampshire, Yorkshire, and Large White. Only one non-synonymous SNP was discovered. This SNP was located on exon 5, and involved a serine to leucine switch.

Table 2. PXR SNP Prevalence Among Swine Breeds

Hanford, Yucatan, Duroc, Pietrain, Yorkshire, and Large White	100% Homozygous Serine
Sinclair	75% Homozygous Serine 25% Heterozygous Serine/Leucine
Hampshire	65% Homozygous Leucine 35% Heterozygous Serine/Leucine

Conclusions

We characterized PXR by generating basic sequence data, analyzing splice variants, creating a tissue expression profile, and screening for SNPs. Basic comparisons show that the pig PXR peptide is more similar to the human than most other possible animal models. Also like the human PXR, our porcine model contains SNPs and splicing variants mostly located in the ligand binding domain. In the future, the information generated about the swine PXR can be further analyzed and used in the assessment of the pig as a suitable pharmacological model.

Acknowledgements

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