

# FXR as a Drug Target to Treat Progressive Familial Intrahepatic Cholestasis

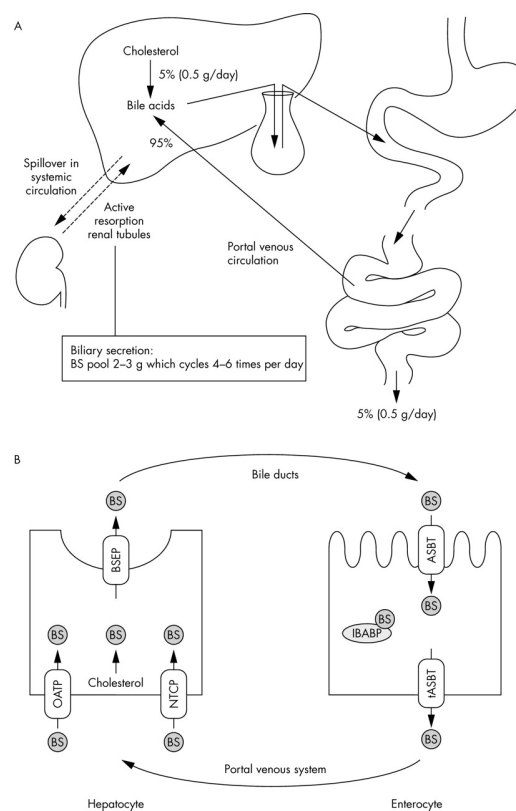
Kathryn Stevens Ivy

College of Arts and Sciences, Vanderbilt University

**Progressive Familial Intrahepatic Cholestasis (PFIC) is a condition that results in the cirrhosis of the liver and eventually liver failure due to impaired bile flow. If left untreated and even if treated, PFIC will result usually in an early death. While the causes of this disease vary, all types present with a similar symptoms and eventual prognosis. There are four main proposed subtypes of intrahepatic cholestasis: disorders of membrane transport and secretion, disorders of bile acid biosynthesis and conjugation, disorders of embryogenesis and lastly, an unclassified group. The careful maintenance of bile salt homeostasis is crucial to the metabolism of fats and normal liver function. These genes are involved with the regulation and transport of bile from hepatocytes into the gallbladder and eventually to the ileum of the small intestines. Because maintenance of symptoms can be regulated to improve longevity and quality of life, it is important to further elucidate the mechanisms that lead to cholestasis. By understanding these mechanisms we can hope to develop treatments that target specifically the precursors and downstream effectors in the previously indicated genes. With early detection of PFIC, there is an opportunity for therapy, however the current pharmacologic opportunities are few and have a low efficacy. Therefore there is much need for a stronger and more selective treatment that can be directed to the cause of PFIC instead of its symptoms. I postulate that FXR is a good target because of its advantageous position as a transcription factor.**

Progressive Familial Intrahepatic Cholestasis (PFIC) is a condition that results in the cirrhosis of the liver and eventually liver failure due to impaired bile flow. If left untreated and even if treated, PFIC will result usually in an early death. While the causes of this disease vary, all types present with similar symptoms and eventual prognosis. There are four main proposed subtypes of intrahepatic cholestasis: disorders of membrane transport and secretion, disorders of bile acid biosynthesis and conjugation, disorders of embryogenesis and lastly, an unclassified group.<sup>1</sup> The careful maintenance of bile salt homeostasis, shown in Fig. 1, is crucial to the metabolism of fats and normal liver function.<sup>4</sup> The basis of the three different classified types is mutation in the gene within the homeostasis pathway. These genes are involved with the regulation and transport of bile from hepatocytes into the gallbladder and eventually to the ileum of the small intestines. It also has been shown that type 1, 2, and 3 of PFIC are autosomal recessive hereditary diseases. The genes that are indicated as the primary cause of these disorders are, respectively, *FIC1*, *BSEP*, and *MDR3*.<sup>2</sup> However, recent evidence suggests most forms of PFIC have a multifactorial genetic basis.<sup>3</sup>

Because maintenance of symptoms can be regulated to improve longevity and quality of life, it

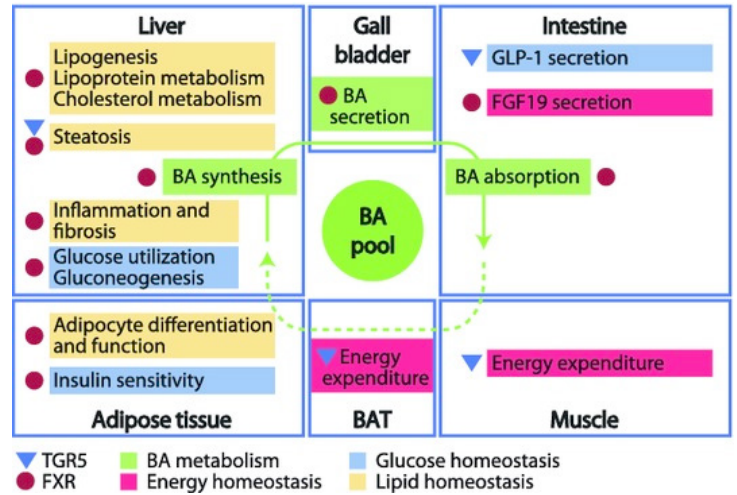


**Fig. 1 shows a schematic of enterohepatic circulation of bile salts by van Mil S W C et al.<sup>4</sup>**

is important to further elucidate the mechanisms that lead to cholestasis. By understanding these mecha-

nisms we can hope to develop treatments that target specifically the precursors and downstream effectors in the previously indicated genes. With early detection of PFIC, there is an opportunity for therapy, however the current pharmacologic opportunities are few and have a low efficacy. Ursodeoxycholic acid (UDCA) is a current treatment. Ursodiol is a secondary biliary acid analog that reduces cholesterol uptake and therefore slows cirrhosis of the liver due to cholestasis.<sup>5</sup> However, few patients respond to such treatment and those who do respond have a mild cessation of cirrhosis. Therefore there is much need for a stronger and more selective treatment that can be directed to the cause of PFIC instead of its symptoms. I postulate that FXR is a good target because of its advantageous position as a transcription factor.

Nuclear Receptors (NRs) are crucial to transcriptional regulation in the cell. In *Homo Sapiens* there are 48 different members of this family that help to control numerous cell processes.<sup>6</sup> In 1995, Wineberger et al. found the ligand of an orphan receptor to be farnesol metabolites. This was the discovery of Farnesoid X-Receptor. Farnesol metabolites are generated by the cell in order to metabolize different steroid hormones, which eventually are metabolized into bile salts.<sup>8, 16</sup> FXR is a NR that dimerizes with its partner Retinoid X-Receptor (RXR) to activate their primary target genes (see Table 1). The overall downstream effect is a strong and fine-tuned



**Fig. 2 gives a graphic display of the importance of FXR in metabolism bile salts and maintaining both glucose, lipid and energy homeostasis.<sup>31</sup>**

metabolize these materials, FXR transcriptional activity recently has been closely linked with metabolic diseases, such as abdominal obesity and Type II diabetes.<sup>9, 11</sup> While these specific conditions are due to an acquired condition, FXR has also been implicated in inherent disorders. This past October, Huang et al. found a strong correlation between the down regulation of FXR and the development of human hepatocellular carcinoma. In a murine model they found that the overexpression of FXR lead to a strong reduction in the size of these tumors.<sup>10</sup>

While there are burgeoning treatments for such diseases that I have mentioned such as modifications to diet and lifestyle to have a healthier life, there are some disorders that are purely genetic. Progressive Familial Intrahepatic Cholestasis is one of these. As mentioned earlier, PFIC leads to the eventual cirrhosis of the liver due to an over presence of bile in the liver because it is not metabolized. While FXR has not been directly implicated in the reasoning behind PFIC, it is logical to examine other trials that have explored the implications of each categorized type of PFIC and see that FXR plays an integral role in the dysfunction of bile salt metabolism in each of these disorders.

Type I PFIC, also known as Byler's Disease, is characterized by hypercholesteremia, low serum and low gamma-glutamyltranspeptidase levels. These symptoms are all thought to be due to a dysregulation of cholesterol and lipid metabolism.<sup>32</sup> Lapunzina et al. has shown a strong correlation in murine models as well as limited clinical models that PFIC type I is due to a mutation in *ATP8B1*, a gene that codes for FIC1, a putative aminophospholipid translocase.<sup>12</sup> These P4-ATPases, also

**Table 1 represents all of the downstream targets of FXR that have been indicated thus far in the literature.<sup>30</sup>**  
\*indicate targets discussed in this paper

KNG1	SLC27A5	PPARA
NROB2	SLC3A1	VLDLR
ABCB11*	APOA1	Fibrinogen
FABP6	APOC2	SDC1
ABCB4*	APOE	VIPR1
FGF19	C3	Alpha-crystallin
ABCC2	PDK4	ATP8B1*
SLCO1B3	PLTP	

regulation of bile homeostasis. Due to this activity it is understandable that FXR is overexpressed in the liver, intestine, kidney and adrenal glands, places where steroid synthesis and metabolism is crucial (see Fig. 2).<sup>7</sup>

While FXR's role seems to be simple, the slightest dysregulation can lead to drastic consequences in bile salt homeostasis. Recently it was shown that FXR is crucial to liver regeneration after a partial hepatectomy and lack of its full activity can lead to the serious condition of carcinogenesis.<sup>7</sup> Since any disruption of FXR activity leads to a subsequent disruption in the lipid and glucose levels due to a dysregulation of the genes that usually

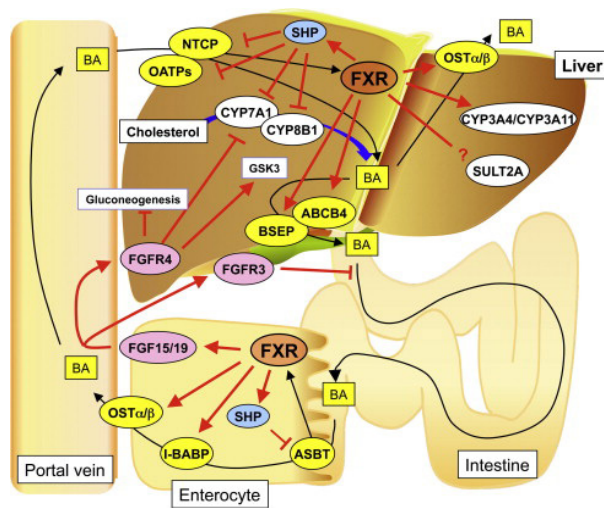
known as flippases, select phospholipids and translocate them from the outer leaflet of the plasma membrane to the inner. They have specific substrates and this leads to the asymmetry of the membrane, which is essential for normal cell function and viability. The lack of this asymmetry leads to a 'leaky' cell since it cannot yield a strong seal on the membrane.<sup>13</sup> While the relationship between FIC1 dysfunction and PFIC is not fully understood, the connection between FXR and transcription of *ATP8B1* is. FXR is upregulated in the presence of bile acids in the enterohepatic system in order to aid in their metabolism, however mutations in FIC1 (gene product of *ATP8B1*) have shown that these bile acids cannot be taken into the hepatocytes and therefore not detected by FXR, their nuclear receptor.<sup>15</sup> This leads to a buildup of bile acids in ducts within liver and therefore cholestasis. The coexpression of FIC1 and FXR is strongly linked not only in the liver and the intestinal track but in numerous homologs throughout other tissues, which implies that FXR is the primary regulator of FIC1 expression.<sup>12,14</sup>

Similar to PFIC1, type II PFIC is characterized by hypercholesterolemia as well as low serum and low gamma-glutamyltranspeptidase levels. However, the etiology of type II is rooted in a malfunction in the lipid and cholesterol transfer through a different protein. ABCB11 is also an aminophospholipid translocator, however its mechanism of action varies slightly. This pump is also referred to the bile-salt excretory pump (BSEP). Unlike FIC1 and other P4-ATPases, these ABC transporters (adenosine triphosphate binding cassette) translocate their substrates in a dimer form rather than a pump form, which is reminiscent of the Na<sup>+</sup>/K<sup>+</sup> ATPase. In type II PFIC, the dysfunction of the pump leads to impaired secretion of bile salts into canaliculi, the bile duct. This leads to a state of cholestasis, similar to type I.<sup>17</sup> Work by both Muller et al. and Suchy et al. has shown that FXR stimulation of bile salts is required for transcriptional control of the *ABCB11*.<sup>18</sup> This feedback mechanism of BSEP pumping bile salts activates FXR, which in turn transcribes more BSEP is crucial and also allows for easy manipulation of the pathway. These disruptions lead to a dysregulation of lipid uptake into hepatocytes and eventually metabolism.

Type III PFIC is characterized by deficiencies in the activity of *MDR3*, another ABC transporter.<sup>32</sup> Unlike BSEP, *MDR3* translocates phosphatidylcholine across the membrane. This is important to the maintenance of the liver because it helps form micelles in

the caniculi that encapsulate bile acids. Until recently, *MDR3* was not recognized as a downstream effector of FXR, but work done by Cui et al. has shown that *MDR3* expression in hepatocytes is highly inducible by exogenously activated FXR, indicating that they are intricately linked. This work was further validated since there is a binding site within the *MDR3* promoter that binds specifically to the FXR/RXR heterodimer to induce transcription. Without this binding event, transcription of *MDR3* gene was shown to not occur.<sup>19</sup>

The relationship between these three types of progressive familial intrahepatic cholestasis seems almost redundant, however regulating the export of steroid derivatives, bile salts, and phospholipids in a coordinated fashion results in the appropriate physiological ratio of these molecules in the bile.<sup>19</sup> It is therefore logical to understand why they are activated by the same heterodimers of transcription factors, FXR/RXR. Unfortunately the treatments for intrahepatic cholestasis are not very well defined because there are many causes that fall in to type IV PFIC, or those with unknown etiology. Such diseases with no specified cause include intrahepatic cholestasis of pregnancy, ARC syndrome, lymphedema-cholestasis syndrome and North American Indian childhood cirrhosis.<sup>20</sup> However, there are possible treatments for type I, II and III due to its solely genetic nature.



**Fig. 3 displays the major roles of FXR in glucose, lipid and energy metabolism. Most of the pathways it is involved in are focused solely on this homeostasis, reducing the concern for cross-reactivity.**

In my research I have shown the strong and well-founded connection between the transcription of the three key elements for type I, II and III (FIC1,



BSEP, and MDR3) and FXR as part of the dimer that promotes their transcription. This element makes FXR a good target for drug therapies in treatment of only type I, II, and III. It is important to recognize that it is only an appropriate candidate for those who have the common and specific mutations that generate these diseases. FXR is highly expressed in the liver and intestines and therefore as a drug target it is valid because its primary responses would only be shown in these organs.<sup>24</sup> However, the nuclear receptor it dimerizes with to elicit a response, retinoid X receptor, has a leveled expression throughout many tissues, including the brain.<sup>25</sup> Therefore, FXR is considered a good target because of its differential expressivity within the body. Recently, further work has been done to elucidate the function of FXR in the rest of the body. Thus far, it has been shown that in other tissues FXR has a different isoform that does not respond to the same agonists, but much more work must be done to validate these claims.<sup>27</sup> To further fine tune the response of this target, consider type II and III PFIC. It has been shown that BSEP and MDR3, mutants in type II and III respectively, are expressed exclusively in the membranes of hepatocytes. Therefore there would be limited side effects in other tissue types with the upregulation of FXR to induce the production of BSEP and MDR3.<sup>21</sup> To further reduce the possibility of cross-reactivity it is important to recognize that FXR has an extremely low binding affinity for bile acids and steroids.<sup>22</sup> Therefore for activation it requires a high concentrations of these ligands; concentrations of this size which are located solely in the enterohepatic system (Fig. 3).<sup>23</sup>

Fig 4

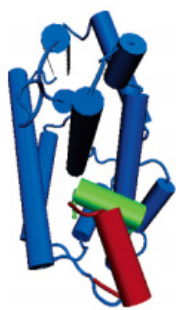
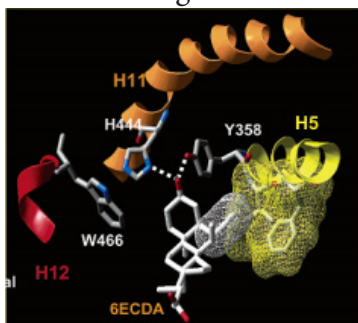


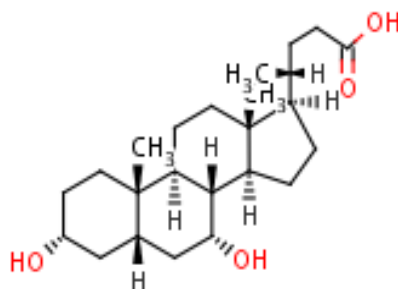
Fig. 5



**Fig. 4 and 5 both show the crystallized structure of FXR. Fig.4 depicts the arrangement of the helices in the activated and inactivated forms. Fig. 5 points out the important residues of FXR.**<sup>27</sup>

Farnesoid X Receptor is the central regulator for bile acid homeostasis, as evident in Fig. 2 and 3. Therefore the majority of its downstream effectors are directly involved in the regulation of bile acid secretion and me-

tabolism.<sup>27</sup> This makes it a strong target to combat PFIC because its involvement in other pathways is small due to the localized expression of its downstream effectors and inducible expression pattern via high concentration of bile salts. Another reason that FXR is a strong drug target is that much is known about the structure and the intricate mechanism of this NR. Fig. 4 and 5 show the intricate details that we have of the structure and binding pattern of FXR.



**Fig. 6 is the molecular structure for CDCA, the ligand for FXR. This depiction was generated using Reaxys.**

It has been shown that the stability and therefore functionality of this nuclear receptor is dependent on the conformation of helix 12 (indicated in the ‘active disposition’ as red and ‘inactive disposition’ as green in Fig. 4).<sup>27</sup> The primary ligand of FXR was identified in 1999 by multiple groups as they indicated that Chenodeoxyholic acid (CDCA; see Fig. 6) was the primary ligand to the orphan receptor, FXR.<sup>16, 28, 29</sup> Very fledgling work has been done to investigate CDCA as a lead compound, however it is logical to assume that more work will be done. Now that the entire binding site has been classified by Rastinejad et al. it can safely be assumed that more studies will be done into optimizing the downstream responses of FXR through its overexpression and activation.

To conclude, the connection between FXR and genetically linked progressive familial intrahepatic cholestasis has clearly been shown by many different sources. It is also advantageous that FXR is the primary TF for all of these proteins because it lends the possibility that one treatment could aid in alleviation of symptoms for Type I, II and III. FXR is a strong candidate for interference in this pathway for the reason that it is so closely linked to the expression of these proteins without other molecules downstream that could give potentially hazardous side effects.

## ENDNOTES

1. Balistreri, William F., Jorge A. Bezerra, Peter Jansen, Saul J. Karpen, Benjamin L. Shneider, and Frederick J. Suchy. “Intrahepatic Cholestasis: Summary of an American Association for the Study of Liver Diseases Single-topic Conference.” *Hepatology* 42.1 (2005): 222-35.
2. Morotti, Raffaella, Frederick Suchy, and Margret Magid. “Progressive Familial Intrahepatic Cholestasis (PFIC) Type 1, 2, and

- 3: A Review of the Liver Pathology Findings.” *Seminars in Liver Disease* 31.01 (2011): 003-10.
3. Sookoian, Silvia, and Carlos J. Pirola. “Genetic Determinants of Acquired Cholestasis: A Systems Biology Approach.” *Frontiers in Bioscience* 17 (2012): 206-10.
4. Van Mil, S W C. “Genetics of Familial Intrahepatic Cholestasis Syndromes.” *Journal of Medical Genetics* 42.6 (2005): 449-63.
5. Jackson, Hannah, Masoud Solaymani-Dodaran, Tim R. Card, Guruprasad P. Aithal, Richard Logan, and Joe West. “Influence of Ursodeoxycholic Acid on the Mortality and Malignancy Associated with Primary Biliary Cirrhosis: A Population-based Cohort Study.” *Hepatology* 46.4 (2007): 1131-137.
6. Olefsky, JM. “Nuclear Receptor minireview series.” *J. Biological Chemistry* 276.40 (2001): 36863-4.
7. Fujino T, Takeuchi A, Maruko-Ohtake A, Ohtake Y, Satoh J, Kobayashi T, Tanaka T, Ito H, Sakamaki R, Kashimura R, Ando K, Nishimaki-Mogami T, Ohkubo Y, Kitamura N, Sato R, Kikugawa K, Hayakawa M. “Critical role of farnesoid X receptor (FXR) for hepatocellular carcinoma cell proliferation.” *Journal of Biochemistry* (Sept. 2012 E Pub ahead of print).
8. Forman, B. “Identification of a Nuclear Receptor That Is Activated by Farnesol Metabolites.” *Cell* 81.5 (1995): 687-93.
9. Kemper, Jongsook Kim. “Regulation of FXR Transcriptional Activity in Health and Disease: Emerging Roles of FXR Cofactors and Post-translational Modifications.” *Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease* 1812.8 (2011): 842-50.
10. Su H, Ma C, Liu J, Li N, Gao M, Huang A, Wang X, Huang W, Huang X. “Downregulation of nuclear receptor FXR is associated with multiple malignant clinicopathological characteristics in human hepatocellular carcinoma.” *Am Journal Physiology and Gastrointestinal Liver Physiology* 2012 Oct. 4. (Epub ahead of print.)
11. Dufer, Martina, Peter Krippeit-Drews, and Gisela. “The Significance of the Nuclear Farnesoid X Receptor in Beta Cell Function.” *Landes Bioscience Journals* 4.5 (2012).
12. Alvarez, L., and Pablo Lapunzina. “Reduced Hepatic Expression of Farnesoid X Receptor in Hereditary Cholestasis Associated to Mutation in ATP8B1.” *Human Molecular Genetics* 13.20 (2004): 2451-460.
13. Siggs, O. M., B. Schnabi, B. Webb, and B. Beutler. “X-linked Cholestasis in Mouse Due to Mutations of the P4-ATPase ATP11C.” *Proceedings of the National Academy of Sciences* 108.19 (2011): 7890-895.
14. Harris, M. “FIC1, a P-type ATPase Linked to Cholestatic Liver Disease, Has Homologues (ATP8B2 and ATP8B3) Expressed throughout the Body.” *Biochimica Et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* 1633.2 (2003): 127-31.
15. Chen, F. “Progressive Familial Intrahepatic Cholestasis, Type 1, Is Associated with Decreased Farnesoid X Receptor Activity.” *Gastroenterology* 126.3 (2004): 756-64.
16. Parks, D. J., S. G. Blanchard, R. K. Bledsoe, G. Chandra, T. G. Consler, S. A. Kliewer, and J. B. Stimmel. “Bile Acids: Natural Ligands for an Orphan Nuclear Receptor.” *Science* 284 (1999): 1365-368.
17. Plass, J. “Farnesoid X Receptor and Bile Salts Are Involved in Transcriptional Regulation of the Gene Encoding the Human Bile Salt Export Pump.” *Hepatology* 35.3 (2002): 589-96.
18. Ananthanarkayanan, M., N. Balasubramanian, M. Makishima, D. J. Mangelsdorf, and F. J. Suchy. “Human Bile Salt Export Pump Promoter Is Transactivated by the Farnesoid X Receptor/bile Acid Receptor.” *Journal of Biological Chemistry* 276.31 (2001): 28857-8865.
19. Huang, L. “Farnesoid X Receptor Activates Transcription of the Phospholipid Pump MDR3.” *Journal of Biological Chemistry* 278.51 (2003): 51085-1090.
20. Van Mil, S W C. “Genetics of Familial Intrahepatic Cholestasis Syndromes.” *Journal of Medical Genetics* 42.6 (2005): 449-63.
21. Teng, Shirley, and Micheline Piquette-Miller. “Regulation of Transporters by Nuclear Hormone Receptors: Implications during Inflammation.” *Molecular Pharmaceutics* 5.1 (2008): 67-76.
22. Buijsman, Rogier C., Pedro H.H. Hermkens, Rachel D. Van Rijn, H. T. Stock, and N. Miranda Teerhuis. “Non-Steroidal Steroid Receptor Modulators.” *Current Medicinal Chemistry* 12.9 (2005): 1017-075.
23. Matsubara, Tsutomu, Fei Li, and Frank J. Gonzalez. “FXR Signaling in the Enterohepatic System.” *Molecular Cell Endocrinology* (2012). [Epub ahead of print]
24. Niu, Yong-dong, Wen Xie, and Wen-xin Qin. “Molecular Mechanism for the Involvement of Nuclear Receptor FXR in HBV-associated Hepatocellular Carcinoma.” *Acta Pharmaceutica Sinica B* 1.2 (2011): 73-79.
25. Arfaoui, A., M. V. Lobo, S. Boulbaroud, A. Quichou, A. Mesfioui, and M. I. Arenas. “Expression of Retinoic Acid Receptors and Retinoid X Receptors in Normal and Vitamin A Deficient Adult Rat Brain.” *Annals of Anatomy* (2012). [Epub ahead of print]
26. Chen, Wei-Dong, Yan-Dong Wang, Lisheng Zhang, Steven Shiah, Meihua Wang, Fan Yang, Donna Yu, Barry M. Forman, and Wendong Huang. “Farnesoid X Receptor Alleviates Age-related Proliferation Defects in Regenerating Mouse Livers by Activating Foxm1b Transcription.” *Hepatology* (2009).
27. Pellicciari, Roberto, Gabriele Costantino, and Stefano Fiorucci. “Farnesoid X Receptor: From Structure to Potential Clinical Applications.” *Journal of Medicinal Chemistry* 48.17 (2005): 5383-403.
28. Makishima, M., A. Y. Okamoto, J. J. Repa, H. Tu, R. M. Learned, A. Luk, M. V. Hull, K. D. Lustig, D. J. Mangelsdorf, and B. Shan. “Identification of a Nuclear Receptor for Bile Acids.” *Science* 284.5418 (1999): 1362-365.
29. Wang, H. “Endogenous Bile Acids Are Ligands for the Nuclear Receptor FXR/BAR.” *Molecular Cell* 3.5 (1999): 543-53.
30. 1H. Liver X receptor-like receptors: Farnesoid X receptor. Last modified on 01/03/2012. Accessed on 12/11/2012. IUPHAR database (IUPHAR-DB), <http://www.iuphar-db.org/DATABASE/ObjectDisplayForward?objectId=603>.
31. Prawitt, Jane, Sandrine Caron, and Bart Staels. “(Bile Acid Metabolism and the Pathogenesis of Type 2 Diabetes.” *Current Diabetes Reports* 11.3 (2011).
32. Morotti, Raffaella, Frederick Suchy, and Margret Magid. “Progressive Familial Intrahepatic Cholestasis (PFIC) Type 1, 2, and 3: A Review of the Liver Pathology Findings.” *Seminars in Liver Disease* 31.01 (2011): 003-10.
33. Mi, Li-Zhi, Srikripa Devarakonda, Joel M. Harp, Qing Han, Roberto Pellicciari, Timothy M. Willson, Sepideh Khorasanizadeh, and Fraydoon Rastinejad. “Structural Basis for Bile Acid Binding and Activation of the Nuclear Receptor FXR.” *Molecular Cell* 11.4 (2003): 1093-100.