Genetic determinants of acquired cholestasis: A systems biology approach

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TABLE OF CONTENTS

1. ABSTRACT

Cholestatic liver diseases encompass a complex spectrum of intrahepatic and cholangiocellular cholestasis, whose etiologies include genetic and environmental components. This review focuses on the role of the genetic component of three adult cholestatic diseases, namely, primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), and intrahepatic cholestasis of pregnancy (ICP). In particular, we integrate genomic, molecular, and physiological data to understand the putative interplay between the underlying genetic mechanisms involved in the susceptibility of these diseases. This approach is based on the hypothesis that a more integrative knowledge of the genetic determinants of cholestatic diseases may have a strong impact on the development of improved therapies. We also propose the strategy of gene prioritization to identity potential candidate genes for disease susceptibility, and show some examples of "leading genes of human cholestatic pathways". Finally, based on the hypothesis that common physiologic processes and molecular networks may influence the risk of adult cholestatic diseases, we used a candidate gene prioritization application based on the use of a protein–protein interaction network as part of the 'interactome'.

2. INTRODUCTION

Human cholestatic liver diseases cover a complex range of intrahepatic and cholangiocellular cholestasis. The etiologies of these diseases include genetic and environmental components, and the triggering-associated factors are diverse, including infections, toxins, and autoimmune processes, among others. Here we examine the role of the genetic component of three adult cholestatic diseases: primary sclerosing cholangitis (PSC), primary biliary cirrhosis (PBC), and intrahepatic cholestasis of pregnancy (ICP). Genomic, molecular, and physiological data are integrated to give us an understanding of the putative interplay between the underlying genetic mechanisms involved in the susceptibility of these clinical pictures. This particular approach is based on the hypothesis that a more integrative knowledge of the genetic determinants of cholestatic diseases may have a strong impact on the development of improved therapies.

While cholangiopathies such as PBC and PSC are progressive diseases, the first one characterized by portal inflammation and immune-mediated destruction of the intrahepatic bile ducts and the second one by inflammation, destruction, and fibrosis of intrahepatic and extrahepatic bile duct, ICP is a relatively benign disorder, at least for the mother. Despite such dramatic clinical and histopathological differences, there are some biological similarities that suggest the involvement of common biological pathways among them (Table 1) (1-3). Hence, our goal is to infer gene networks that capture common physiological pathways among the three diseases and predict new candidate genes potentially associated with them.

3. SYSTEMS BIOLOGY APPROACH, AND MECHANISTIC AND THERAPEUTIC INSIGHTS

3.1. Evidence of multifactorial genetic basis

As shown in Table 1, the three cholestatic diseases with which we will deal here show evidence of heritability and familial clustering. In addition, DNA variation in either candidate genes or signals identified by genome-wide association studies (GWAS) has been regarded as susceptibility or modifier variants of these disorders. Nevertheless, like in other complex diseases, the associated gene variants have small or moderate effects on disease risk. This picture is in part explained by the nature of the complex disorder itself and the strong impact of environmental factors working in concert with the genetic influence. In this regard, the influence of epigenetic factors, such as DNA methylation and histone modifications, remain totally unexplored, but it is very likely that they play a major role in the disease etiology.

In addition, while these diseases have worldwide distribution, their incidence is strongly variable according to the different ethnic groups, suggesting the involvement of population-specific genetic risk factors. This is supported by the observation that some gene variants are associated with disease susceptibility in some but not in all populations. For example,

gene variants of cytotoxic T-lymphocyte antigen 4 (CTLA-4, also known as CD152CTLA-4) were associated with PBC in populations from United Kingdom (4), United States (5), and China (6), but the findings were not replicated in the Italian population (7), in a combined study from the United Kingdom and northern Italy (8), or in the Brazilian population (9). Similarly, a 32-base pair deletion in the chemokine receptor 5 gene (CCR5-D32), expressed on T-cells and macrophages, shows conflicting results about a putative association with susceptibility to PSC. In Australian (10) and Belgian populations (11), but not in Scandinavian ones (12), the variant was associated either with susceptibility or progression of the disease.

Interestingly, even in the presence of different mechanisms for the induction of cholestasis in PSC, PBC, and ICP, some genes are reported as being associated with all of them (Figure 1). The most remarkable example is *ABCB4* (also known as *MDR3*), a member of the superfamily of ATP-binding cassette (ABC) transporters. *ABCB4* is a liver-specific membrane transporter of phosphatidylcholine – a major component of the bile, from the inner to the outer leaflet of the canalicular membrane (13); the biliary phospholipid excretion is a critical mechanism to protect plasma membranes of cholangiocytes from bile salts.

Mutations of this gene lead to progressive familial intrahepatic cholestasis type 3; the gene is also regarded as *PFIC3*. As mentioned before, gene variants of *ABCB4* were associated with ICP (14), and also were regarded as modifiers of disease progression in PBC or predictors of survival to liver transplantation in PSC (15). Interestingly, another family member of the ABC transporter superfamily, MRP2 (*ABCC2*), was found to be associated with ICP (16).

Besides the relevant data about the function of *ABCB4*, it is also important to note from a clinical point of view that most patients with *ABCB4* deficiency have a favorable outcome with ursodeoxycholic acid (UDCA) therapy (17). Hence, *ABCB4* has been proposed as good candidate for a targeted pharmacologic approach (17).

Moreover, *ABCB4* mutations also represent a genetic factor involved in the presence of cholesterol gallstone disease in adults (18), a condition also associated with the three cholestatic diseases. Additional evidence from human and animal studies supporting the role of *ABCB4* as a "cholestatic-candidate gene" is shown in Table 2.

3.2. Systems biology-based approaches and gene-regulatory networks in the etiology of PSC, PBC, and ICP

A second explanation for the relatively small effect attributed to the associated gene variants in the three cholestatic disorders analyzed here is that neither the common SNPs nor the rare mutations identified so far as putative susceptibility genes are necessarily the "causative agents" by themselves but they act as networks of genes that interact among each other. Then, it seems very unlikely that the complex gene-gene and gene-environment interactions that characterize PSC, PBC, and ICP can be easily explained or even modeled in a transgenic model of disease. Hence, system biology-based approaches based on the combinations of certain data, *e.g.*, expression array analysis, the measurement of transcript levels, the relationships between transcript levels and clinical traits, etc., might be able to address such complex interactions.

Systems biology introduces a new and integrative concept of the pathogenesis of human disorders, and suggests the presence of common physiologic processes and molecular networks influencing the risk of a disease. The significance of systems biology-based strategy and a "proof-of-concept" about the potential application of this novel strategy was previously shown (19, 20). Here, we show a model of this concept for the explanation of the genetic determinants of the three cholestatic diseases.

An example of this approach is the prediction of integrated associations and gene networks, derived from several resources such as a collection of PubMed abstracts, high-throughput proteomic screening, and microarray profiling. Figure 2 shows a putative gene network for the *ABCB4* gene. Interestingly, *ABCB4* interacts closely with four genes that may explain its pleiotropic effects, namely, *ABCB1*, *ABCB7*, *HAX1*, and *NR1H4* (*FXR*).

The observation on the impact of the putative gene network of *ABCB4* on the pathogenesis of the above-mentioned cholestatic disorders fits also with the association between gene variants of the nuclear bile acid receptor *NR1H4* (*FXR*) and susceptibility to ICP (21, 22), PSC (23), and gallstone disease (24) (Figure 2).

The second remarkable gene reported as a susceptibility gene of the three diseases is the nuclear receptor subfamily 1, group I, member 2 (*NR1I2, PXR, SXR*), a nuclear receptor that binds and is activated by a variety of endogenous and xenobiotic compounds. *PXR* is also involved in the detoxification of cholestatic bile acids, and in the regulation of the expression of genes involved in the biosynthesis transport and metabolism of bile acids. *PXR* activates the transcription of multiple genes involved in the metabolism and secretion of potentially harmful, exogenous and endogenous compounds. Interestingly, *PXR* is activated by pregnanes and by the toxic bile acid lithocholic acid. In addition, *PXR* binds to a response element in the promoters of the *CYP3A4* and *ABCB1/MDR1* genes. Functional gene variants in *PXR* were not only associated with susceptibility to ICP (25), but also appear to modify disease course in PSC (26). Finally, endogenous and exogenous PXR activators are used to treat pruritus in chronic inflammatory liver diseases such as PBC (27). A gene network for *PXR* (*NR1I2, SXR)* based on data of PubMed abstracts, high-throughput proteomic screening, and microarray profiling is shown in Figure 3. A gene that encodes a UDP-glucuronosyltransferase (*UGT1A1,* also known as bilirubin UDP-glucuronosyltransferase isozyme 1) is predicted to be co-expressed and also up-regulated by PXR; *UGT1A1* transforms small lipophilic molecules, such as steroids, bilirubin, hormones, and drugs, into water-soluble, excretable metabolites. *UGT1A1* plays a major role in controlling serum levels of bilirubin by contributing to its conjugation with glucuronic acid, a key detoxification step of this potentially neurotoxic compound.

3.3. Identifying potential candidate genes for disease susceptibility based on the strategy of gene prioritization

While the phenotypic manifestations of the adult cholestatic diseases are readily characterized, the underling genetic component is far more difficult to understand. Some progress, however, has been made for the understanding of PSC and PBC since the era of the GWAS. For instance, a GWAS on PSC showed strong HLA-associations near the *HLA-B* at chromosome *6p21,* and also with a subset of genes involved in bile homeostasis and inflammatory pathways (a genetic region surrounding the *GPC6* locus) (28). Similarly, common genetic variants at the HLA class II, IL12A, and IL12RB2 loci were reported as

significantly associated with PBC from a genome-wide association analysis including DNA samples from 2072 Canadian and U.S. subjects (29).

Despite the thorough scanning of thousands of markers across the complete human genome during the post-genome era, only a small proportion of the genetic component of the adult cholestatic diseases, namely, PSC, PBC, and ICP, can be explained by either common gene variants or gene mutations. However, this observation is not restricted to human cholestatic diseases; on the contrary, it is a common finding in the genetic of complex diseases. In addition, GWAS generate large sets of potential candidate genes, with the identification of the most likely disease-related ones being extremely difficult, in part owing to the need for multiple testing adjustments to avoid false positives but at the cost of imposing a really high statistical cutoff.

Some strategies have been proposed to improve the identification of susceptibility genes based on computational tools for prioritization of candidate genes, by assuming that the majority of causative genes are functionally closely related from the functional point of view. This approach has the advantage of not only facilitating the identification of a disease-associated gene, but it may also help to elucidate the disease biology.

To accomplish this purpose, we used three different strategies. First, we looked for sets of functionally related genes in combinations of loci associated with ICP, PBC, and PSC, on the basis of all the available data about either candidate gene association studies or GWAS, when available for each disease. Following this premise, we used the bioinformatic tool *Prioritizer*, available at http://pcdoeglas.med.rug.nl/prioritizer/, in which the basic premise for ranking candidate genes is positional candidate gene prioritization. Briefly, this application uses a Bayesian approach to generate a gene network based upon data from Gene Ontology, KEGG, BIND, HPRD, and Reactome, a dataset that contains approximately 70,000 predicted protein-protein interactions, 3,000 predicted human protein-protein interactions and co-expression data, derived from approximately 10,000 human microarray experiments stored within the Gene Expression Omnibus and the Stanford Microarray Database (30). As a result, this interaction network ranks the best positional candidates genes on the basis of their interactions, assuming that the causative ones for any disorder will be involved in only a few different biological pathways (30).

Using the *Prioritizer* program we investigated the putative biological relationships among all the genetic loci that have been reported until now in association with ICP, PBC, and PSC; *Prioritizer* input (training sets) for the three diseases is shown in Figures 4, 5, and 6, respectively. The application of *Prioritizer* to the three adult cholestatic diseases revealed a complex biological network containing several genes operating in interesting related pathways. For example, for genes related to ICP, *Prioritizer* analysis revealed a putative gene network associated with *ABCC2* composed of *NFKβ* (nuclear factor of kappa light polypeptide gene enhancer in B-cells 2), a transcription factor involved in many biological processes such as inflammation, immunity, differentiation, cell growth, tumorigenesis, and apoptosis, and *HIF1α* (hypoxia inducible factor 1α), a transcription factor found in mammalian cells cultured under reduced oxygen tension that plays an essential role in cellular and systemic homeostatic responses to hypoxia. Hypoxic liver injury, even at molecular level, was not related before to the pathogenesis of ICP. Nevertheless, severe hypoxic injury was reported in a variant of hepatic sickle cell crisis associated with intrahepatic cholestasis, which has severe jaundice and high transaminase levels (31). Thus, one may speculate that some level of insufficient hepatic perfusion in women suffering from ICP may occur.

In the case of PBC, both *CASP8* (caspase 8, apoptosis-related cysteine peptidase), involved in the programmed cell death induced by Fas and various apoptotic stimuli, and *CD28*, a T-cell-specific surface glycoprotein, composed the CTLA4-associated gene network. Finally, for the PSC-associated genes, the *Prioritizer* analysis revealed a putative gene network associated with *HLA-DRB2* composed of *PSMB8* (proteasome subunit, beta type, 8 that plays critical roles in major histocompatibility complex class I antigen processing) and *BRD2* (bromodomain containing 2, a protein serine/threonine kinase having distinct roles in initiating apoptosis; the dotted distribution pattern in nucleus may be a morphologic marker of cell apoptosis).

The second approach we used was gene prioritization through genomic data fusion by the ENDEAVOUR program (32). ENDEAVOUR is a software application for the computational prioritization of candidate genes underlying biological processes or diseases, based on their similarity to known genes involved in a disease (32). The hypothesis underlying the gene prioritization procedure by ENDEAVOUR is that test genes (candidates) are ranked based on their similarity with a set of known training genes. Briefly, ENDEAVOUR makes use of statistics to compute a ranking of test genes according to their similarity to the training genes. In a subsequent step, these rankings are integrated into a single ranking by making use of order statistics. The ranking of the test genes is built by ENDEAVOUR after integrating the data into a mathematical model based on the similarity of the test genes with the training genes. Vector-based data are scored by the Pearson correlation between a test profile and the training average, whereas attribute-based data are scored by Fisher's omnibus analysis on statistically over-represented training attributes (32). We used the same list of training genes as we had used for prioritization by the *Prioritizer* program (all the genes already known to be involved in the genetic susceptibility of ICP, PSC, and PBC).

Based on the description above, Table 3 lists the first 50 prioritized genes out of 23.712 from the whole human genome with a significant association with the ICP training set. Similar analyses are shown for PSC and PBC in Table 4 and Table 5, respectively. Interestingly, gene prioritization by ENDEAVOUR showed that some gene families might be regarded as involved in the genetic susceptibility to the adult cholestatic diseases. For instance, the Cytochrome P450 (CYP) superfamily, represented by almost 13 genes, was prioritized with high significance levels for ICP. As already known, the function of most CYP enzymes is to catalyze the oxidation of organic substances, including metabolic intermediates such as lipids and steroidal hormones, as well as xenobiotic substances such as drugs and other toxic chemicals. There are just few studies in the Chinese population that have evaluated the association between gene variants of the CYP enzymes and the obstetric cholestasis (33, 34), so that replication in other populations deserves to be performed.

In the case of PSC, the family of chemokine receptors was significantly over-represented, suggesting that they may play a significant role in the etiology of this disorder. An interesting hypothesis about pathogenetic similarities between PSC and arteriosclerosis was previously formulated, suggesting that PSC represents "arteriosclerosis of the bile duct" initiated by toxic biliary lipids (35). In this scenario, adhesion molecules like ICAMs, and chemokine receptors may explain the pathobiology of the bile ductular lesions.

The signal transducer and activator of transcription (STAT) genes deserve particular attention regarding their putative role in the genetic susceptibility of PBC. STAT proteins are involved in several functions, including the maintenance of immune tolerance, tumor surveillance, and apoptosis. There is no evidence for the association between gene variants in any STAT genes and PBC as yet, and hence additional studies are warranted to investigate these candidate genes.

A final and remarkable observation involves a group of genes prioritized for the three disorders that overlap each other with significant *p* values, namely, genes belonging to the nuclear receptors family (Figure 7). ENDEAVOUR prioritized several susceptibility loci that reveal a molecular link between the three disorders. Hence, the complex network shared by ICP, PSC, and PBS includes the thyroid hormone receptor alpha (*THRA*), liver X receptor α and β (*LXRA* and *LXRB*), constitutive androstane receptor (*CAR*), retinoic acid receptor (*RARB)*, hepatocyte nuclear factor 4α (*HNF4A*), and *RAC3*. None of them were considered before as candidate genes, while their role in the bile acid homeostasis and cholestatic liver injury is unquestionable (36). The complex transcriptional and post-transcriptional regulations exerted by these nuclear receptors make them promising therapeutic targets for cholestatic liver diseases (37). List of references about candidate gene association studies is shown in Table 6.

Based on the hypothesis that common physiologic processes and molecular networks may influence the risk of adult cholestatic diseases, we proposed as a final approach for candidate gene prioritization: the use of a protein–protein interaction network (PPIN) or 'interactome'. For this purpose, we used the open web bioinformatic resource *ToppGene Suite* (http://toppgene.cchmc.org) to detect functional enrichment of our gene list based on Transcriptome, Proteome, Regulome (TFBS and miRNA), Ontologies (GO, Pathway), Phenotype (human disease and mouse phenotype), Pharmacome (Drug-Gene associations), and literature co-citation (38). Briefly, the protein–protein interaction network-based disease candidate gene prioritization uses social and Web networks analysis algorithms (extended versions of the PageRank and HITS algorithms, and the K-Step Markov method) (38).

To build the candidate gene list, we used the same training set of genes as those mentioned above for ICP, PBC, and PSC. By the *ToppFun* application that relies on gene annotations and sequence features, namely, GO: Molecular Function, GO: Biological Process, Mouse Phenotype, Pathways, Protein Interactions, Protein Domains, transcription factor-binding sites, miRNA-target genes, disease-gene associations, drug-gene interactions, and Gene Expression, we observed again that the common functional pathway shared by ICP, PBC, and PSC is the one enlisted as Nuclear Receptors in Lipid Metabolism and Toxicity (identification source: CGAP BioCarta, h_nuclearRsPathway, *p* value = $1.191E^{-11}$) (Figure 8). This pathway is notably ranked first for the three diseases, suggesting that nuclear receptors play a critical role in the physiopathology of adult cholestatic diseases, regardless of their etiology and clinical presentation. Interestingly, the *ToppFun* application also predicts drug-gene interactions based on data and annotations deposited in databases, such as The Comparative Toxicogenomics Database (CTD), Drugbank, Metador (Manually Annotated Targets and Drugs Online Resource), and Stitch (search tool for interactions of chemicals). For example, CTD is able to predict which genes/proteins interact with a chemical, and as shown in Table 7, based on the list of training genes we used for this analysis, ursodeoxycholic acid was predicted for ICP, PBC, and PSC. Hence, the rationale for its use is biologically plausible as supported by the clinical indication in the three diseases and successful treatment response. STITCH, a resource to explore known and predicted interactions of chemicals and proteins, predicted another chemical for ICP and PSC, 7alpha-hydroxy-4-cholesten-3-one, which was ranked with high significant *p* values. Previous reports showed that serum concentrations of 7alpha-hydroxy-4-cholesten-3-one (alpha-HC) may serve as a convenient marker for the semiquantitative assessment of bile acid synthesis (39).

As a final observation, the application predicted a miRNA: the hsa-miR-148a for ICP and PSC (*p* value = $1.637E^{-3}$); miRNAs are post-transcriptional regulators that bind to complementary sequences in the 3' UTRs of target mRNAs, usually resulting in gene silencing. This miRNA was recently regarded as a modulator the inflammation-associated cytokine interleukin-6 and oncogenesis in cholangiocarcinoma (40).

4. PERSPECTIVE

The genetic component of human adult cholestatic disorders such as ICP, PSC, and PBC is still under elucidation. Integrative knowledge in genetics by different approaches such as systems biology is becoming a powerful instrument to identify not only associated candidate genes but also common disease-associated molecular pathways. These liver disorders need a deeper analysis of DNA sequence variations and their interactions in concert with liver expression patterns and transcript abundance. Gene prioritization is gaining acceptance for modeling and performing integrative strategies, and some examples of "leading genes of human cholestatic pathways" were shown. Finally a comprehensive examination of each phenotype, analyzing its similarities and differences, may also impact on the design of more effective therapeutic strategies. A clear example of this is the fact that UDCA treatment is the most commonly used pharmacological intervention for ICP and PBC. Nevertheless, although clinical and biochemical response to this drug is remarkable for both disorders, the underlying physiopathological mechanism of ICP and PBC is, at least until now, regarded as absolutely different.

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6. REFERENCES

1. M. M. Kaplan and M. E. Gershwin: Primary biliary cirrhosis. *N Engl J Med* 353, 1261-1273 (2005)

2. Y. M. Lee and M. M. Kaplan: Primary sclerosing cholangitis. *N Engl J Med* 332, 924-933 (1995)

3. B. Pathak, L. Sheibani and R. H. Lee: Cholestasis of pregnancy. *Obstet Gynecol Clin North Am* 37, 269-282 (2010)

4. K. Agarwal, D. E. Jones, A. K. Daly, O. F. James, B. Vaidya, S. Pearce and M. F. Bassendine: CTLA-4 gene polymorphism confers susceptibility to primary biliary cirrhosis. *J Hepatol* 32, 538-541 (2000)

5. B. D. Juran, E. J. Atkinson, E. M. Schlicht, B. L. Fridley and K. N. Lazaridis: Primary biliary cirrhosis is associated with a genetic variant in the 3' flanking region of the CTLA4 gene. *Gastroenterology* 135, 1200-1206 (2008)

6. L. Y. Fan, X. Q. Tu, Q. B. Cheng, Y. Zhu, R. Feltens, T. Pfeiffer and R. Q. Zhong: Cytotoxic T lymphocyte associated antigen-4 gene polymorphisms confer susceptibility to primary biliary cirrhosis and autoimmune hepatitis in Chinese population. *World J Gastroenterol* 10, 3056-3059 (2004)

7. S. Oertelt, T. P. Kenny, C. Selmi, P. Invernizzi, M. Podda and M. E. Gershwin: SNP analysis of genes implicated in T cell proliferation in primary biliary cirrhosis. *Clin Dev Immunol* 12, 259-263 (2005)

8. P. Donaldson, S. Veeramani, A. Baragiotta, A. Floreani, C. Venturi, S. Pearce, V. Wilson, D. Jones, O. James, J. Taylor, J. Newton and M. Bassendine: Cytotoxic T-lymphocyte-associated antigen-4 single nucleotide polymorphisms and haplotypes in primary biliary cirrhosis. *Clin Gastroenterol Hepatol* 5, 755-760 (2007)

9. P. L. Bittencourt, S. A. Palacios, E. L. Cancado, G. Porta, F. J. Carrilho, A. A. Laudanna, J. Kalil and A. C. Goldberg: Cytotoxic T lymphocyte antigen-4 gene polymorphisms do not confer susceptibility to autoimmune hepatitis types 1 and 2 in Brazil. *Am J Gastroenterol* 98, 1616-1620 (2003)

10. R. Eri, J. R. Jonsson, N. Pandeya, D. M. Purdie, A. D. Clouston, N. Martin, D. Duffy, E. E. Powell, J. Fawcett, T. H. Florin and G. L. Radford-Smith: CCR5-Delta32 mutation is strongly associated with primary sclerosing cholangitis. *Genes Immun* 5, 444-450 (2004)

11. L. Henckaerts, J. Fevery, S. W. Van, C. Verslype, P. Yap, F. Nevens, T. Roskams, L. Libbrecht, P. Rutgeerts and S. Vermeire: CC-type chemokine receptor 5-Delta32 mutation protects against primary sclerosing cholangitis. *Inflamm Bowel Dis* 12, 272-277 (2006)

12. E. Melum, T. H. Karlsen, U. Broome, E. Thorsby, E. Schrumpf, K. M. Boberg and B. A. Lie: The 32-base pair deletion of the chemokine receptor 5 gene (CCR5-Delta32) is not associated with primary sclerosing cholangitis in 363 Scandinavian patients. *Tissue Antigens* 68, 78-81 (2006)

13. P. J. Meier and B. Stieger: Bile salt transporters. *Annu Rev Physiol* 64, 635-661 (2002)

14. P. H. Dixon and C. Williamson: The molecular genetics of intrahepatic cholestasis of pregnancy. *Obstet Med* 1, 65-71 (2008)

15. T. H. Karlsen and J. R. Hov: Genetics of cholestatic liver disease in 2010. *Curr Opin Gastroenterol* 26, 251-258 (2010)

16. S. Sookoian, G. Castano, A. Burgueno, T. F. Gianotti and C. J. Pirola: Association of the multidrug-resistance-associated protein gene (ABCC2) variants with intrahepatic cholestasis of pregnancy. *J Hepatol* 48, 125-132 (2008)

17. A. Davit-Spraul, E. Gonzales, C. Baussan and E. Jacquemin: The spectrum of liver diseases related to ABCB4 gene mutations: pathophysiology and clinical aspects. *Semin Liver Dis* 30, 134-146 (2010)

18. O. Rosmorduc, B. Hermelin and R. Poupon: MDR3 gene defect in adults with symptomatic intrahepatic and gallbladder cholesterol cholelithiasis. *Gastroenterology* 120, 1459-1467 (2001)

19. A. S. Azmi, Z. Wang, P. A. Philip, R. M. Mohammad and F. H. Sarkar: Proof of concept: network and systems biology approaches aid in the discovery of potent anticancer drug combinations. *Mol Cancer Ther* 9, 3137-3144 (2010)

20. S. Sookoian and C. J. Pirola: Metabolic Syndrome: From the Genetics to the Pathophysiology. *Curr Hypertens Rep* (2010). Epub (DOI: 10.1007/s11906-010-0164-9).

21. S. W. Van Mil, A. Milona, P. H. Dixon, R. Mullenbach, V. L. Geenes, J. Chambers, V. Shevchuk, G. E. Moore, F. Lammert, A. G. Glantz, L. A. Mattsson, J. Whittaker, M. G. Parker, R. White and C. Williamson: Functional variants of the central bile acid sensor FXR identified in intrahepatic cholestasis of pregnancy. *Gastroenterology* 133, 507-516 (2007)

22. V. Zimmer, R. Mullenbach, E. Simon, C. Bartz, S. Matern and F. Lammert: Combined functional variants of hepatobiliary transporters and FXR aggravate intrahepatic cholestasis of pregnancy. *Liver Int* 29, 1286-1288 (2009)

23. L. Henckaerts, M. Jaspers, S. W. Van, L. Vliegen, J. Fevery, H. Nuytten, T. Roskams, P. Rutgeerts, J. J. Cassiman, S. Vermeire and H. Cuppens: Cystic fibrosis transmembrane conductance regulator gene polymorphisms in patients with primary sclerosing cholangitis. *J Hepatol* 50, 150-157 (2009)

24. P. Kovacs, R. Kress, J. Rocha, U. Kurtz, J. F. Miquel, F. Nervi, N. Mendez-Sanchez, M. Uribe, H. H. Bock, R. Schirin-Sokhan, M. Stumvoll, J. Mossner, F. Lammert and H. Wittenburg: Variation of the gene encoding the nuclear bile salt receptor FXR and gallstone susceptibility in mice and humans. *J Hepatol* 48, 116-124 (2008)

25. G. Castano, A. Burgueno, G. T. Fernandez, C. J. Pirola and S. Sookoian: The influence of common gene variants of the xenobiotic receptor (PXR) in genetic susceptibility to intrahepatic cholestasis of pregnancy. *Aliment Pharmacol Ther* 31, 583-592 (2010)

26. T. H. Karlsen, B. A. Lie, F. K. Frey, E. Thorsby, U. Broome, E. Schrumpf and K. M. Boberg: Polymorphisms in the steroid and xenobiotic receptor gene influence survival in primary sclerosing cholangitis. *Gastroenterology* 131, 781-787 (2006)

27. K. Wallace, D. E. Cowie, D. K. Konstantinou, S. J. Hill, T. E. Tjelle, A. Axon, M. Koruth, S. A. White, H. Carlsen, D. A. Mann and M. C. Wright: The PXR is a drug target for chronic inflammatory liver disease. *J Steroid Biochem Mol Biol* 120, 137-148 (2010)

28. T. H. Karlsen, A. Franke, E. Melum, A. Kaser, J. R. Hov, T. Balschun, B. A. Lie, A. Bergquist, C. Schramm, T. J. Weismuller, D. Gotthardt, C. Rust, E. E. Philipp, T. Fritz, L. Henckaerts, R. K. Weersma, P. Stokkers, C. Y. Ponsioen, C. Wijmenga, M. Sterneck, M. Nothnagel, J. Hampe, A. Teufel, H. Runz, P. Rosenstiel, A. Stiehl, S. Vermeire, U. Beuers, M. P. Manns, E. Schrumpf, K. M. Boberg and S. Schreiber: Genome-wide association analysis in primary sclerosing cholangitis. *Gastroenterology* 138, 1102-1111 (2010)

29. G. M. Hirschfield, X. Liu, C. Xu, Y. Lu, G. Xie, Y. Lu, X. Gu, E. J. Walker, K. Jing, B. D. Juran, A. L. Mason, R. P. Myers, K. M. Peltekian, C. N. Ghent, C. Coltescu, E. J. Atkinson, E. J. Heathcote, K. N. Lazaridis, C. I. Amos and K. A. Siminovitch: Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N Engl J Med* 360, 2544-2555 (2009)

30. L. Franke, B. H. van, L. Fokkens, E. D. de Jong, M. Egmont-Petersen and C. Wijmenga: Reconstruction of a functional human gene network, with an application for prioritizing positional candidate genes. *Am J Hum Genet* 78, 1011-1025 (2006)

31. S. Banerjee, C. Owen and S. Chopra: Sickle cell hepatopathy. *Hepatology* 33, 1021-1028 (2001)

32. S. Aerts, D. Lambrechts, S. Maity, L. P. Van, B. Coessens, S. F. De, L. C. Tranchevent, M. B. De, P. Marynen, B. Hassan, P. Carmeliet and Y. Moreau: Gene prioritization through genomic data fusion. *Nat Biotechnol* 24, 537-544 (2006)

33. X. Wang, L. Zhang, R. Q. Ou, J. Wang, S. Y. Liu, Q. Chen and P. C. Su: [Association between CYP1A1 gene polymorphism and intrahepatic cholestasis of pregnancy]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 25, 70-72 (2008)

34. L. Zhang, S. Y. Liu, Q. Chen, Q. Y. Shi, H. Zou and L. Wu: [Association between polymorphisms of CYP17 and CYP3A4 genes and intrahepatic cholestasis of pregnancy in Chengdu]. *Sichuan Da Xue Xue Bao Yi Xue Ban* 37, 551-3, 582 (2006)

35. P. Fickert, T. Moustafa and M. Trauner: Primary sclerosing cholangitis--the arteriosclerosis of the bile duct? *Lipids Health Dis* 6, 3 (2007)

36. M. Arreseand S. J. Karpen: Nuclear receptors, inflammation, and liver disease: insights for cholestatic and fatty liver diseases. *Clin Pharmacol Ther* 87, 473-478 (2010)

37. G. Zollnerand M. Trauner: Nuclear receptors as therapeutic targets in cholestatic liver diseases. *Br J Pharmacol* 156, 7-27 (2009)

38. J. Chen, E. E. Bardes, B. J. Aronow and A. G. Jegga: ToppGene Suite for gene list enrichment analysis and candidate gene prioritization. *Nucleic Acids Res* 37, W305-W311 (2009)

39. G. Sauter, F. Berr, U. Beuers, S. Fischer and G. Paumgartner: Serum concentrations of 7alpha-hydroxy-4-cholesten-3-one reflect bile acid synthesis in humans. *Hepatology* 24, 123-126 (1996)

40. C. Braconi, N. Huang and T. Patel: MicroRNA-dependent regulation of DNA methyltransferase-1 and tumor suppressor gene expression by interleukin-6 in human malignant cholangiocytes. *Hepatology* 51, 881-890 (2010)

41. K. Said, H. Glaumann and A. Bergquist: Gallbladder disease in patients with primary sclerosing cholangitis. *J Hepatol* 48, 598-605 (2008)

42. D. J. Brandt, R. L. MacCarty, J. W. Charboneau, N. F. LaRusso, R. H. Wiesner and J. Ludwig: Gallbladder disease in patients with primary sclerosing cholangitis. *AJR Am J Roentgenol* 150, 571-574 (1988)

43. J. A. Summerfield, E. Elias, G. D. Hungerford, V. L. Nikapota, R. Dick and S. Sherlock: The biliary system in primary biliary cirrhosis. A study by endoscopic retrograde cholangiopancreatography. *Gastroenterology* 70, 240-243 (1976)

44. A. Ropponen, R. Sund, S. Riikonen, O. Ylikorkala and K. Aittomaki: Intrahepatic cholestasis of pregnancy as an indicator of liver and biliary diseases: a population-based study. *Hepatology* 43, 723-728 (2006)

45. Y. Popov, E. Patsenker, P. Fickert, M. Trauner and D. Schuppan: Mdr2 (Abcb4)-/- mice spontaneously develop severe biliary fibrosis via massive dysregulation of pro- and antifibrogenic genes. *J Hepatol* 43, 1045-1054 (2005)

46. K. E. Nakken, S. Nygard, T. K. Haaland, K. E. Berge, A. Odegaard, K. J. Labori and M. G. Raeder: Gene expression profiles reflect sclerosing cholangitis activity in abcb4 (-/-) mice. *Scand J Gastroenterol* 44, 211-218 (2009)

47. O. Rosmorduc, B. Hermelin, P. Y. Boelle, R. Parc, J. Taboury and R. Poupon: ABCB4 gene mutation-associated cholelithiasis in adults. *Gastroenterology* 125, 452-459 (2003)

48. J. F. Lucena, J. I. Herrero, J. Quiroga, B. Sangro, J. Garcia-Foncillas, N. Zabalegui, J. Sola, M. Herraiz, J. F. Medina and J. Prieto: A multidrug resistance 3 gene mutation causing cholelithiasis, cholestasis of pregnancy, and adulthood biliary cirrhosis. *Gastroenterology* 124, 1037-1042 (2003)

49. E. Jacquemin, J. M. De Vree, D. Cresteil, E. M. Sokal, E. Sturm, M. Dumont, G. L. Scheffer, M. Paul, M. Burdelski, P. J. Bosma, O. Bernard, M. Hadchouel and R. P. Elferink: The wide spectrum of multidrug resistance 3 deficiency: from neonatal cholestasis to cirrhosis of adulthood. *Gastroenterology* 120, 1448-1458 (2001)

50. A. Floreani, I. Carderi, D. Paternoster, G. Soardo, F. Azzaroli, W. Esposito, A. Variola, A. M. Tommasi, D. Marchesoni, C. Braghin and G. Mazzella: Intrahepatic cholestasis of pregnancy: three novel MDR3 gene mutations. *Aliment Pharmacol Ther* 23, 1649-1653 (2006)

51. E. Jacquemin, D. Cresteil, S. Manouvrier, O. Boute and M. Hadchouel: Heterozygous non-sense mutation of the MDR3 gene in familial intrahepatic cholestasis of pregnancy. *Lancet* 353, 210-211 (1999)

52. M. L. Eloranta, T. Hakli, M. Hiltunen, S. Helisalmi, K. Punnonen and S. Heinonen: Association of single nucleotide polymorphisms of the bile salt export pump gene with intrahepatic cholestasis of pregnancy. *Scand J Gastroenterol* 38, 648-652 (2003)

53. R. Mullenbach, K. J. Linton, S. Wiltshire, N. Weerasekera, J. Chambers, E. Elias, C. F. Higgins, D. G. Johnston, M. I. McCarthy and C. Williamson: ABCB4 gene sequence variation in women with intrahepatic cholestasis of pregnancy. *J Med Genet* 40, e70 (2003)

54. M. L. Eloranta, J. T. Heiskanen, M. J. Hiltunen, A. J. Mannermaa, K. R. Punnonen and S. T. Heinonen: Multidrug resistance 3 gene mutation 1712delT and estrogen receptor alpha gene polymorphisms in Finnish women with obstetric cholestasis. *Eur J Obstet Gynecol Reprod Biol* 105, 132-135 (2002)

55. A. Floreani, I. Carderi, D. Paternoster, G. Soardo, F. Azzaroli, W. Esposito, A. Variola, A. M. Tommasi, D. Marchesoni, C. Braghin and G. Mazzella: Intrahepatic cholestasis of pregnancy: three novel MDR3 gene mutations. *Aliment Pharmacol Ther* 23, 1649-1653 (2006)

56. R. Mullenbach, A. Bennett, N. Tetlow, N. Patel, G. Hamilton, F. Cheng, J. Chambers, R. Howard, S. D. Taylor-Robinson and C. Williamson: ATP8B1 mutations in British cases with intrahepatic cholestasis of pregnancy. *Gut* 54, 829-834 (2005)

57. J. G. Mella, E. Roschmann, J. C. Glasinovic, A. Alvarado, M. Scrivanti and B. A. Volk: Exploring the genetic role of the HLA-DPB1 locus in Chileans with intrahepatic cholestasis of pregnancy. *J Hepatol* 24, 320-323 (1996)

58. M. L. Eloranta, J. Heiskanen, M. Hiltunen, S. Helisalmi, A. Mannermaa and S. Heinonen: Apolipoprotein E alleles in women with intrahepatic cholestasis of pregnancy. *Scand J Gastroenterol* 35, 966-968 (2000)

59. J. Laasanen, S. Helisalmi, S. Iivonen, M. L. Eloranta, M. Hiltunen and S. Heinonen: Gamma 2 actin gene (enteric type) polymorphism is not associated with obstetric cholestasis or preeclampsia. *Fetal Diagn Ther* 23, 36-40 (2008)

60. R. Poupon, C. Ping, Y. Chretien, C. Corpechot, O. Chazouilleres, T. Simon, S. C. Heath, F. Matsuda, R. E. Poupon, C. Housset and V. Barbu: Genetic factors of susceptibility and of severity in primary biliary cirrhosis. *J Hepatol* 49, 1038-1045 (2008)

61. P. Invernizzi, C. Selmi, F. Poli, S. Frison, A. Floreani, D. Alvaro, P. Almasio, F. Rosina, M. Marzioni, L. Fabris, L. Muratori, L. Qi, M. F. Seldin, M. E. Gershwin and M. Podda: Human leukocyte antigen polymorphisms in Italian primary biliary cirrhosis: a multicenter study of 664 patients and 1992 healthy controls. *Hepatology* 48, 1906-1912 (2008)

62. G. M. Hirschfield, X. Liu, C. Xu, Y. Lu, G. Xie, Y. Lu, X. Gu, E. J. Walker, K. Jing, B. D. Juran, A. L. Mason, R. P. Myers, K. M. Peltekian, C. N. Ghent, C. Coltescu, E. J. Atkinson, E. J. Heathcote, K. N. Lazaridis, C. I. Amos and K. A. Siminovitch: Primary biliary cirrhosis associated with HLA, IL12A, and IL12RB2 variants. *N Engl J Med* 360, 2544-2555 (2009)

63. X. Liu, P. Invernizzi, Y. Lu, R. Kosoy, Y. Lu, I. Bianchi, M. Podda, C. Xu, G. Xie, F. Macciardi, C. Selmi, S. Lupoli, R. Shigeta, M. Ransom, A. Lleo, A. T. Lee, A. L. Mason, R. P. Myers, K. M. Peltekian, C. N. Ghent, F. Bernuzzi, M. Zuin, F. Rosina, E. Borghesio, A. Floreani, R. Lazzari, G. Niro, A. Andriulli, L. Muratori, P. Muratori, P. L. Almasio, P. Andreone, M. Margotti, M. Brunetto, B. Coco, D. Alvaro, M. C. Bragazzi, F. Marra, A. Pisano, C. Rigamonti, M. Colombo, M. Marzioni, A. Benedetti, L. Fabris, M. Strazzabosco, P. Portincasa, V. O. Palmieri, C. Tiribelli, L. Croce, S. Bruno, S. Rossi, M. Vinci, C. Prisco, A. Mattalia, P. Toniutto, A. Picciotto, A. Galli, C. Ferrari, S. Colombo, G. Casella, L. Morini, N. Caporaso, A. Colli, G. Spinzi, R. Montanari, P. K. Gregersen, E. J. Heathcote, G. M. Hirschfield, K. A. Siminovitch, C. I. Amos, M. E. Gershwin and M. F. Seldin: Genome-wide meta-analyses identify three loci associated with primary biliary cirrhosis. *Nat Genet* 42, 658-660 (2010)

64. G. M. Hirschfield, X. Liu, Y. Han, I. P. Gorlov, Y. Lu, C. Xu, Y. Lu, W. Chen, B. D. Juran, C. Coltescu, A. L. Mason, P. Milkiewicz, R. P. Myers, J. A. Odin, V. A. Luketic, D. Speiciene, C. Vincent, C. Levy, P. K. Gregersen, J. Zhang, E. J. Heathcote, K. N. Lazaridis, C. I. Amos and K. A. Siminovitch: Variants at IRF5-TNPO3, 17q12-21 and MMEL1 are associated with primary biliary cirrhosis. *Nat Genet* 42, 655-657 (2010)

65. Y. Ohishi, M. Nakamura, N. Iio, S. Higa, M. Inayoshi, Y. Aiba, A. Komori, K. Omagari, H. Ishibashi and K. Tsukamoto: Single-nucleotide polymorphism analysis of the multidrug resistance protein 3 gene for the detection of clinical progression in Japanese patients with primary biliary cirrhosis. *Hepatology* 48, 853-862 (2008)

66. A. Tanaka, S. Nezu, S. Uegaki, K. Kikuchi, A. Shibuya, H. Miyakawa, S. Takahashi, I. Bianchi, P. Zermiani, M. Podda, H. Ohira, P. Invernizzi and H. Takikawa: Vitamin D receptor polymorphisms are associated with increased susceptibility to primary biliary cirrhosis in Japanese and Italian populations. *J Hepatol* 50, 1202-1209 (2009)

67. L. P. Lakatos, E. Bajnok, D. Hegedus, T. Toth, P. Lakatos and F. Szalay: Vitamin D receptor, oestrogen receptor-alpha gene and interleukin-1 receptor antagonist gene polymorphisms in Hungarian patients with primary biliary cirrhosis. *Eur J Gastroenterol Hepatol* 14, 733-740 (2002)

68. T. H. Karlsen, K. M. Boberg, M. Vatn, A. Bergquist, J. Hampe, E. Schrumpf, E. Thorsby, S. Schreiber and B. A. Lie: Different HLA class II associations in ulcerative colitis patients with and without primary sclerosing cholangitis. *Genes Immun* 8, 275-278 (2007)

69. A. Spurkland, S. Saarinen, K. M. Boberg, S. Mitchell, U. Broome, L. Caballeria, E. Ciusani, R. Chapman, G. Ercilla, O. Fausa, I. Knutsen, A. Pares, F. Rosina, O. Olerup, E. Thorsby and E. Schrumpf: HLA class II haplotypes in primary sclerosing cholangitis patients from five European populations. *Tissue Antigens* 53, 459-469 (1999)

70. K. Wiencke, T. H. Karlsen, K. M. Boberg, E. Thorsby, E. Schrumpf, B. A. Lie and A. Spurkland: Primary sclerosing cholangitis is associated with extended HLA-DR3 and HLA-DR6 haplotypes. *Tissue Antigens* 69, 161-169 (2007)

71. X. Yang, S. N. Cullen, J. H. Li, R. W. Chapman and D. P. Jewell: Susceptibility to primary sclerosing cholangitis is associated with polymorphisms of intercellular adhesion molecule-1. *J Hepatol* 40, 375-379 (2004)

72. M. Trauner, P. Fickert and M. Wagner: MDR3 (ABCB4) defects: a paradigm for the genetics of adult cholestatic syndromes. *Semin Liver Dis* 27, 77-98 (2007)

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Figure 1. Genetic determinants of adult acquired cholestasis: overlapping candidate genes reported in association with ICP, PSC, and PBC, and shared molecular pathways PSC: primary sclerosing cholangitis; PBC: primary biliary cirrhosis; ICP: intrahepatic cholestasis of pregnancy. HLA: Human leukocyte antigen MHC: Major histocompatibility complex

Figure 2. Putative gene network for the *ABCB4* gene: in silico prediction of integrated associations derived from several resources, including a collection of PubMed abstracts, high-throughput proteomic screening, and microarray profiling (Gene Network at http://www.sabiosciences.com/genenetwork/genenetworkcentral.php). ABCB1: ATP-binding cassette, sub-family B (MDR/TAP), member 1 (aliases P-gp, CD243, GP170, ABC20, MDR1). ABCB7: ATP-binding cassette, sub-family B (MDR/TAP), member 7 (aliases EST140535, Atm1p, ASAT, MRP). HAX1: HCLS1 associated protein X-1 (aliases HCLS1 (and PKD2) associated protein). NR1H1: (aliases EcRH).

Figure 3. Putative gene network for the *NR1I2 (PXR)* gene: in silico prediction of integrated associations derived from several resources, including a collection of PubMed abstracts, high-throughput proteomic screening, and microarray profiling (Gene Network at http://www.sabiosciences.com/genenetwork/genenetworkcentral.php). NR1I2: nuclear receptor subfamily 1, group I, member 2. NCOR2: nuclear receptor corepressor 2. POU1F1: POU class 1 homeobox 1. UGT1A1: UDP glucuronosyltransferase 1 family, polypeptide A1. ABCB1: ATP-binding cassette, sub-family B (MDR/TAP), member 1. CYP24A1: cytochrome P450, family 24, subfamily A, polypeptide 1. CYP3A4: cytochrome P450, family 3, subfamily A, polypeptide 4. CYP3A: cytochrome P450, family 3, subfamily A. HNF4A: hepatocyte nuclear factor 4, alpha. NCOA1: nuclear receptor coactivator 1.

Figure 4. Gene-network analysis of the genes highlighted by the *Prioritizer* bioinformatic tool for intrahepatic cholestasis of pregnancy (ICP). Susceptibility loci were defined around 16 known ICP disease-associated susceptibility genes (list in the right bottom corner; the list includes either candidate genes previously reported to be associated with ICP or genes ranked based on their similarity with the set of candidates genes considering Gene Ontology + Microarray Co-Expression + Protein-Protein Interactions). The colors indicate the locus with which they are related.

Figure 5. Gene-network analysis of genes highlighted by the *Prioritizer* bioinformatic tool for primary biliary cirrhosis (PBC). Susceptibility loci were defined around 14 known PBC disease-associated susceptibility genes (list in the right bottom corner, the list includes either candidate genes previously reported to be associated with PBC or genes ranked based on their similarity with the set of candidates genes considering Gene Ontology + Microarray Co-Expression + Protein-Protein Interactions). The colors indicate the locus with which they are related.

Figure 6. Gene-network analysis of the genes highlighted by the *Prioritizer* bioinformatic tool for primary sclerosing cholangitis (PSC). Susceptibility loci were defined around eight known PSC disease-associated susceptibility genes (list in the right bottom corner; the list includes either candidate genes previously reported to be associated with PSC or genes ranked based on their similarity with the set of candidates genes considering Gene Ontology + Microarray Co-Expression + Protein-Protein Interactions). The colors indicate the locus with which they are related.

Figure 7. *ENDEAVOUR* combined analysis of the three adult-cholestatic susceptibility loci. Figure shows the results of the gene prioritization built by the *ENDEAVOUR* bioinformatic tool from the whole human genome (23.712 genes) for intrahepatic cholestasis of pregnancy, primary biliary cirrhosis, and primary sclerosing cholangitis *Overlapping genes for ICP, PBC, and PSC: List of Approved Gene Symbol and Names* ABCB1: ATP-binding cassette, sub-family B (MDR/TAP), member 1 (MDR1). ABCC3: ATP-binding cassette, sub-family C (CFTR/MRP), member 3 (MRP3). CD4: CD4 molecule. CD74: CD74 molecule, major histocompatibility complex, class II invariant chain. GHR: growth hormone receptor. HLA-A/ HLA-6: major histocompatibility complex, class I, A/. HLA-DMA: major histocompatibility complex, class II, DM alpha. HLA-DOA: major histocompatibility complex, class II, DO a. HLA-DRA: major histocompatibility complex, class II, DR alpha. HLA-DRB3: major histocompatibility complex, class II, DR beta 3. HLA-DRB5: major histocompatibility complex, class II, DR beta 5. HNF4A: hepatocyte nuclear factor 4, alpha. THRA: thyroid hormone receptor, alpha. NCOA3: nuclear receptor coactivator 3

(RAC3), NR0B2: nuclear receptor subfamily 0, group B, member 2. NR1H2: nuclear receptor subfamily 1, group H, member 2 (LXRb). NR1H3: nuclear receptor subfamily 1, group H, member 3 (LXRa). NR1I3: nuclear receptor subfamily 1, group I, member 3 (CAR). PTPRC: protein tyrosine phosphatase, receptor type, C. RARB: retinoic acid receptor, beta. TAP2: transporter 2, ATP-binding cassette, sub-family B (MDR/TAP). *Overlapping genes for ICP and PBC: List of Approved Gene Symbol and Names* ABCA12: ATP-binding cassette, sub-family A (ABC1), member 12. ABCA3: ATP-binding cassette, sub-family A (ABC1), member 3. ABCB6: ATP-binding cassette, sub-family B (MDR/TAP), member 6. ABCC1/4/5/6: ATP-binding cassette, sub-family C member 1 to 6. ABCD2: ATP-binding cassette, sub-family D (ALD), member 2/4. ABCG1: ATP-binding cassette, sub-family G (WHITE), member 1. ESRR: estrogen-related receptor alpha, beta, gamma. GC: glucocorticoid receptor. NR2E3: nuclear receptor subfamily 2, group E, member 3. NR4A1: nuclear receptor subfamily 4, group A, member 1. NR5A1/A2: nuclear receptor subfamily 5, group A, member 1 and 2. PDGFRA: platelet-derived growth factor receptor, alpha polypeptide. PPARA/ PPARD/PPARG: peroxisome proliferator-activated receptors (PPARs) alpha, delta and gamma. RARA: retinoic acid receptor, alpha and gamma. RXRA: retinoid X receptor, alpha, beta, gamma /B/G/ THR: thyroid hormone receptor, beta. *Overlapping genes for ICP and PSC: List of Approved Gene Symbol and Names* ADRB2: adrenergic, beta-2-, receptor, surface. B2M: beta-2-microglobulin. C8A: complement component 8, alpha polypeptide. CD1D: CD1d molecule. CFTR: cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7). FCGRT: Fc fragment of IgG, receptor, transporter, alpha. FGB: fibrinogen beta chain. SLC10A1: solute carrier family 10 (sodium/bile acid cotransporter family), member 1. SLC2A2: solute carrier family 2 (facilitated glucose transporter), member 2. TAP1: transporter 1, ATP-binding cassette, sub-family B (MDR/TAP). TAPBP: TAP binding protein (tapasin). TAPBPL: TAP binding protein-like. *Overlapping genes for PBC and PSC: List of Approved Gene Symbol and Names* ABCC2: ATP-binding cassette, sub-family C (CFTR/MRP), member 2. CD28: CD28 molecule. CD86: CD86 molecule. CSF3R: colony stimulating factor 3 receptor (granulocyte). ESR2: estrogen receptor 2 (ER beta). IL2RG: interleukin 2 receptor, gamma. IL4R: interleukin 4 receptor. TNFRSF1: tumor necrosis factor receptor superfamily, member 1A.

Figure 8. Candidate gene prioritization based on functional enrichment analysis and protein-protein interaction network. *ToppFunEnriched* results for ICP, PBC, and PSC. Disease pathways analysis was based on combined data from KEGG, BioCarta, BioCyc, Reactome, GenMAPP, and MSigDB.

Table 1. Human acquired intrahepatic cholestatic liver diseases (PSC, PBC and ICP): Clinical characteristics, histological and progressive course, and biological similarities

	PSC	PBC	ICP
Worldwide prevalence and geographical distribution	Most prevalent in northern Europe (higher in Nordic countries)	Most prevalent in northern Europe	Most prevalence in South America, particularly Chile (Araucanian Indian descent), Scandinavia, and South Asia
Sexual dimorphism	Affects men more commonly than women	Affects primarily women 9:1 ratio	Affects pregnant women
Evidence of associated immune mechanisms	-Probable, but not conclusive -Presence of anti-neutrophil cytoplasmic antibody	-Immune-mediated destruction of the intrahepatic bile ducts -Presence of antimitochondrial antibodies	No evidence
Common presenting symptoms	Initially asymptomatic; itching, fatigue and jaundice in advance disease	Fatigue and pruritus	Pruritus
Evidence of heritability	-Familial occurrence was reported. -First-degree relatives and siblings of PSC patients show higher prevalence.	-Most common in first-degree relatives of patients (at least one affected family member, with mother–daughter and sister–sister combinations) -High concordance rate among monozygotic twins	Significant familial clustering
Coexisting co-morbidities	- Inflammatory bowel disease (most often ulcerative colitis) -Retroperitoneal or mediastinal fibrosis.	Sjögren's syndrome and scleroderma. Interstitial pneumonitis, sarcoidosis, renal tubular acidosis	Probably associated with other pregnancy-related disorders
Incidence of gallstones	High (41, 42)	High (more than 39%) (43)	High (more than 10%) (44)
Clinical course	Chronic cholestatic disorder	Chronic cholestatic disorder	- Self-limited disease - Complete resolution following delivery, but with high recurrence rates in subsequent pregnancies
Altered lipid profiles	No evidence	Hyperlipidemia and hypercholesterolemia	No conclusive information
Outcome of liver injury without intervention	Cirrhosis, portal hypertension, and liver failure	Cirrhosis, portal hypertension, and liver failure	Self-limited
Prognosis and mortality	Median survival is 12 years from diagnosis.	Overall median survival ranges between 10 and 15 years.	- Benign course for the mother - High incidence of fetal complications.
Treatment response to UDCA	Its efficacy has not been demonstrated.	Very effective, recommended therapy	Very effective, recommended therapy
Abnormalities in the composition of bile acids	Probable altered pattern of lithocholic acid (LCA)	-Probable increased formation of sulfated bile acids -Elevated levels of cholic acid than chenodeoxycholic acid -Altered urinary bile acid pattern: cholic acid:chenodeoxycholic acid ratio of 1.6	-Altered cholic acid (CA) / chenodeoxycholic acid (CDCA) -Deoxycholic acid (DCA) is markedly diminished. - Altered urinary bile acid pattern: high increase in CA and CDCA, and decreased Excretion of DCA and LCA
Role of associated infection	- Suspected bacterial products acting as toxic proinflammatory agents -Cytomegalovirus	-Elevated incidence of urinary tract infections (Escherichia coli) -Other suspected agents: Chlamydia, Novosphingobium aromaticivorans, and lactobacilli	- Increased incidence of urinary tract infection -Increased incidence of hepatitis C infection
Evidence of increased gut permeability	Yes: suggested chronic portal bacteremia and toxic bile acid metabolites produced by enteric flora	Yes: suggested increased permeability of tight and retention of endotoxin	Yes: suggested leaky gut that enhances the absorption of bacterial endotoxin
Role of environmental exposure	Probably, cigarette smoking	Exposure to environmental chemicals (halogenated hydrocarbons)	-Low dietary selenium intake -Seasonal variation reported

PSC: primary sclerosing cholangitis; PBC: primary biliary cirrhosis; ICP: intrahepatic cholestasis of pregnancy (1-3).

Table 2. Summary of findings supporting *ABCB4* (*MDR3*) as "cholestatic-candidate gene"

Findings	Reference
Abcb4−/− mouse suffers from a cholestatic syndrome with a histological picture resembling that of PSC in humans, including liver fibrosis.	(45)
Abcb4 (-/-) mice secrete phosphatidylcholine-deficient bile and develop sclerosing cholangitis (SC).	(46)
Individuals with mutations in *ABCB4* are particularly prone to cholesterol gallstone formation.	(47)
Mutations in the *ABCB4* gene were reported in patients with symptoms of PBC with negative antimitochondrial antibodies.	(48)
Fifty percent of the patients with PFIC may benefit from treatment with UDCA.	(49)
MDR3 mutations were associated with intrahepatic cholestasis of pregnancy.	(50, 51)

Table 3. The gene prioritization built by ENDEAVOUR for intrahepatic cholestasis of pregnancy: List of the first 50 prioritized candidate genes from the whole human genome (23.712 genes) with a significant association with the training set.

Approved Gene Symbol	Approved Gene Name	P value
Major histocompatibility complex family		
HLA-DRB5	Major histocompatibility complex, class II, DR beta 5	1,62E-13
HLA-DRA	Major histocompatibility complex, class II, DR alpha	1,38E-12
HLA-DQB1	Major histocompatibility complex, class II, DQ beta 1	4,22E-11
HLA-DOA	Major histocompatibility complex, class II, DO alpha	5,45E-11
HLA-DMA	Major histocompatibility complex, class II, DM alpha	6,31E-11
HLA-DQA2	Major histocompatibility complex, class II, DQ alpha 2	7,75E-11
HLA-DOB	Major histocompatibility complex, class II, DO beta	1,91E-08
HLA-B	Major histocompatibility complex, class I, B	2,35E-08
HLA-DRB3	Major histocompatibility complex, class II, DR beta 3	4,13E-08
CD74	CD74 molecule, major histocompatibility complex, class II invariant chain	1,01E-09
Cytochrome P450, family		
CYP3A5	Cytochrome P450, family 3, subfamily A, polypeptide 5	2,23E-09
CYP3A43	Cytochrome P450, family 3, subfamily A, polypeptide 43	2,80E-09
CYP3A7	Cytochrome P450, family 3, subfamily A, polypeptide 7	4,44E-09
CYP1A2	Cytochrome P450, family 1, subfamily A, polypeptide 2	3,00E-08
CYP39A1	Cytochrome P450, family 39, subfamily A, polypeptide 1	8,63E-08
CYP2C9	Cytochrome P450, family 2, subfamily C, polypeptide 9	1,35E-07
CYP8B1	Cytochrome P450, family 8, subfamily B, polypeptide 1	1,91E-07
CYP1B1	Cytochrome P450, family 1, subfamily B, polypeptide 1	2,46E-07
CYP27A1	Cytochrome P450, family 27, subfamily A, polypeptide 1	4,90E-07
CYP2B6	Cytochrome P450, family 2, subfamily B, polypeptide 6	5,40E-07
CYP2D6	Cytochrome P450, family 2, subfamily D, polypeptide 6	6,24E-07
CYP11A1	Cytochrome P450, family 11, subfamily A, polypeptide 1	8,30E-07
CYP2C18	Cytochrome P450, family 2, subfamily C, polypeptide 18	1,15E-06
Transcription factors and nuclear receptors		
HNF4A	Hepatocyte nuclear factor 4, alpha	6,99E-09
PPARA	Peroxisome proliferator-activated receptor alpha	1,43E-08
NR1H3	Nuclear receptor subfamily 1, group H, member 3	1,60E-08
NR2F1	Nuclear receptor subfamily 2, group F, member 1	3,15E-07
NR1I3	Nuclear receptor subfamily 1, group I, member 3	2,19E-08
NR2F6	Nuclear receptor subfamily 2, group F, member 6	1,22E-07
NR0B2	Nuclear receptor subfamily 0, group B, member 2	5,22E-10
NR5A2	Nuclear receptor subfamily 5, group A, member 2	7,10E-07
PPARG	Peroxisome proliferator-activated receptor gamma	6,62E-07
RARB	Retinoic acid receptor, beta	1,00E-06
Apolipoproteins and genes involved in controlling HDL cholesterol, HDL structure, and delivery of cholesterol to steroidogenic tissues		
APOA1	Apolipoprotein A-I	2,60E-08
APOA2	Apolipoprotein A-II	1,05E-07
APOH	Apolipoprotein H (beta-2-glycoprotein I)	3,87E-07
SCARB1	Scavenger receptor class B, member 1	3,58E-07
PON1	Paraoxonase 1	6,54E-07
ATP-binding cassette family		
ABCB1	ATP-binding cassette, sub-family B (MDR/TAP), member 1	3,99E-07
ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	9,42E-07
ABCC6	ATP-binding cassette, sub-family C (CFTR/MRP), member 6	1,11E-06
Hormone-receptors		
PGR	Progesterone receptor	5,64E-08
ESR1	Estrogen receptor 1	1,57E-07
Other		
ALB	Albumin	1,01E-07
B2M	Beta-2-microglobulin	2,61E-07
C8A	Complement component 8, alpha polypeptide	2,66E-07
FGB	Fibrinogen beta chain	4,35E-07
HPX	Hemopexin	5,74E-07
SLC10A1	Solute carrier family 10 (sodium/bile acid cotransporter family), member 1	7,37E-07

Table 4. The gene prioritization built by ENDEAVOUR for primary sclerosing cholangitis: List of the first 50 prioritized candidate genes from the whole human genome (23.712 genes) with a significant association with the training set.

Approved Gene Symbol	Approved Gene Name	P value
Major histocompatibility complex family		
HLA-DRB5	Major histocompatibility complex, class II, DR beta 5	3,70E-13
HLA-A|HLA-G	Major histocompatibility complex, class I, A/G	2,14E-12
HLA-E	Major histocompatibility complex, class I, E	7,89E-12
HLA-DRA	Major histocompatibility complex, class II, DR alpha	3,21E-10
HLA-DQA2	Major histocompatibility complex, class II, DQ alpha 2	1,04E-08
HLA-DMA	Major histocompatibility complex, class II, DM alpha	1,04E-09
HLA-DPA1	Major histocompatibility complex, class II, DP alpha 1	2,50E-09
HLA-DOA	Major histocompatibility complex, class II, DO alpha	3,58E-09
HLA-DQB1	Major histocompatibility complex, class II, DQ beta 1	5,21E-08
HLA-DQA1	Major histocompatibility complex, class II, DQ alpha 1	8,17E-08
HLA-F	Major histocompatibility complex, class I, F	1,56E-07
HLA-DRB3	Major histocompatibility complex, class II, DR beta 3	6,13E-07
HLA-DPB1	Major histocompatibility complex, class II, DP beta 1	9,86E-07
CD74	CD74 molecule, major histocompatibility complex, class II invariant chain	2,03E-09
Immunological response, adhesion molecule, chemokine receptor		
LILRB1	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 1	9,36E-07
CD28	CD28 molecule	6,72E-07
CD1A	CD1a molecule	1,43E-06
CXCR4	Chemokine (C-X-C motif) receptor 4	6,49E-10
CCR1	Chemokine (C-C motif) receptor 1	2,59E-08
CCR2	Chemokine (C-C motif) receptor 2	1,29E-09
ICAM3	Intercellular adhesion molecule 3	5,92E-10
ICAM2	Intercellular adhesion molecule 2	4,94E-09
CD4	CD4 molecule	3,88E-08
CD1D	CD1d molecule	1,49E-07
CCR4	Chemokine (C-C motif) receptor 4	1,51E-07
CCR8	Chemokine (C-C motif) receptor 8	4,76E-07
CCRL1	Chemokine (C-C motif) receptor-like 1	1,38E-06
CCBP2	Chemokine binding protein 2	1,02E-06
CCR3	Chemokine (C-C motif) receptor 3	7,24E-08
KIR3DL1	Killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 1	5,03E-07
Transcription factors and nuclear receptors		
NR1H3	Nuclear receptor subfamily 1, group H, member 3	6,37E-08
HNF4A	Hepatocyte nuclear factor 4, alpha	1,70E-07
NR1I3	Nuclear receptor subfamily 1, group I, member 3	6,47E-07
NR4A2	Nuclear receptor subfamily 4, group A, member 2	6,79E-07
NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	1,73E-06
NR0B2	Nuclear receptor subfamily 0, group B, member 2	8,94E-08
Other		
HFE	Hemochromatosis	1,97E-07
PTPRC	Protein tyrosine phosphatase, receptor type, C	3,16E-07
CSF3R	Colony stimulating factor 3 receptor (granulocyte)	5,15E-07
TAP1	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	5,38E-07
B2M	Beta-2-microglobulin	4,40E-09
AFP	Alpha-fetoprotein	6,63E-07
ITGB2	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)	7,22E-07
ITGAX	Integrin, alpha X (complement component 3 receptor 4 subunit)	1,33E-06
TAP2	Transporter 2, ATP-binding cassette, sub-family B (MDR/TAP)	3,93E-08
SELL	Selectin L	1,10E-06
FGB	Fibrinogen beta chain	1,21E-06
ITGAL	Integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide	8,49E-09
FAS	Fas (TNF receptor superfamily, member 6)	1,49E-06

Table 5. The gene prioritization built by ENDEAVOUR for primary biliary cirrhosis: List of the first 50 prioritized candidate genes from the whole human genome (23.712 genes) with a significant association with the training set.

Approved Gene Symbol	Approved Gene Name	P value
Immunological response, adhesion molecule.		
CD86	CD86 molecule	5,75E-09
IL12B	Interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)	3,88E-12
CD80	CD80 molecule	1,37E-08
IL2RG	Interleukin 2 receptor, gamma	1,70E-07
IL12RB1	Interleukin 12 receptor, beta 1	9,09E-07
IRF3	Interferon regulatory factor 3	1,54E-06
IRF1	Interferon regulatory factor 1	4,80E-07
CSF3R	Colony stimulating factor 3 receptor (granulocyte)	5,73E-07
Signal transducer and activator of transcription		
STAT1	Signal transducer and activator of transcription 1, 91kDa	8,37E-09
STAT5A	Signal transducer and activator of transcription 5A	7,78E-08
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	7,87E-08
STAT6	Signal transducer and activator of transcription 6, interleukin-4 induced	1,25E-07
STAT5B	Signal transducer and activator of transcription 5B	3,61E-07
Transcription factors and nuclear receptors		
NR1H4	Nuclear receptor subfamily 1, group H, member 4	8,15E-10
NR3C1	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)	8,98E-09
NCOA3	Nuclear receptor coactivator 3	2,63E-08
NR0B2	Nuclear receptor subfamily 0, group B, member 2	6,29E-08
HNF4A	Hepatocyte nuclear factor 4, alpha	2,34E-08
PPARA	Peroxisome proliferator-activated receptor alpha	4,06E-09
RXRA	Retinoid X receptor, alpha	9,32E-08
NR4A1	Nuclear receptor subfamily 4, group A, member 1	1,39E-07
RARA	Retinoic acid receptor, alpha	1,70E-07
NR4A2	Nuclear receptor subfamily 4, group A, member 2	2,01E-07
NR1H2	Nuclear receptor subfamily 1, group H, member 2	4,86E-07
NR1H3	Nuclear receptor subfamily 1, group H, member 3	4,95E-07
NR2C2	Nuclear receptor subfamily 2, group C, member 2	5,62E-07
NR2F1	Nuclear receptor subfamily 2, group F, member 1	5,77E-07
NR5A1	Nuclear receptor subfamily 5, group A, member 1	8,48E-07
RXRG	Retinoid X receptor, gamma	1,22E-06
NR0B1	Nuclear receptor subfamily 0, group B, member 1	3,34E-07
PPARG	Peroxisome proliferator-activated receptor gamma	8,73E-07
RARB	Retinoic acid receptor, beta	4,75E-07
Hormone-receptors		
PGR	Progesterone receptor	2,24E-10
ESR2	Estrogen receptor 2 (ER beta)	1,19E-09
ESRRB	Estrogen-related receptor beta	6,05E-07
ESRRA	Estrogen-related receptor alpha	5,87E-08
ESRRG	Estrogen-related receptor gamma	1,16E-07
THRB	Thyroid hormone receptor, beta	1,84E-07
THRA	Thyroid hormone receptor, alpha	1,49E-09
Major histocompatibility complex family		
HLA-DRA	Major histocompatibility complex, class II, DR alpha	9,87E-07
HLA-DRB5	Major histocompatibility complex, class II, DR beta 5	1,33E-07
ATP-binding cassette family		
ABCC3	ATP-binding cassette, sub-family C (CFTR/MRP), member 3	3,39E-07
ABCC2	ATP-binding cassette, sub-family C (CFTR/MRP), member 2	3,81E-07
ABCG2	ATP-binding cassette, sub-family G (WHITE), member 2	9,02E-07
ABCD4	ATP-binding cassette, sub-family D (ALD), member 4	1,51E-06
Other		
SP1	Sp1 transcription factor	8,42E-07
LIFR	Leukemia inhibitory factor receptor alpha	1,33E-06
CREBBP	CREB binding protein	1,79E-07
PTPRC	Protein tyrosine phosphatase, receptor type, C	2,03E-07

Table 6. Reference list of candidate genes with previous evidence about association with intrahepatic cholestasis of pregnancy, primary biliary cirrhosis and primary sclerosing cholangitis

Locus	References
Intrahepatic cholestasis of pregnancy	
ABCC2	(16)
ABCB11	(52)
ABCB4	(48, 53-55)
ATP8B1	(56)
NR1H4	(21)
HLA DPB1	(57)
APOE	(58)
HLA DPA1	(57)
ESR2	(54)
CYP3A4	(34)
ACTG2	(59)
CYP1A1	(33)
NR1I2	(25)
Primary biliary cirrhosis	
CTLA4	(4-6, 60)
HLADRB2	(61, 62)
IL12A	(62-64)
IL12RB2	(62)
IRF5	(63, 64)
STAT4	(62)
ABCB1	(60)
NR1I2	(60)
SLCA2	(60)
SLC11A1	(60)
ABCB4	(48, 60, 65)
VDR	(66, 67)
ESR1	(67)
Primary sclerosing cholangitis	
HLA DRB	(28, 68-70)
GPC5	(28, 71)
CCR5	(10, 11)
ABCB4	(72)
NR1I2	(26)
ICAM1	(71)

Table 7. ToppFun Enriched results for ICP, PBC, and PSC: Prediction of drug-gene interactions.

ID	Drug	Source	P-value
Intrahepatic cholestasis of pregnancy			
D014580	Ursodeoxycholic Acid	CTD	1.052E-11
CID000000334	7alpha-hydroxy-4-cholesten-3-one	Stitch	1.268E-11
D012293	Rifampin	CTD	5.610E-11
D013656	Taurocholic acid	CTD	1.216E-10
CID000005645	Ursodeoxycholic acid	Stitch	2.330E-10
Primary biliary cirrhosis			
C065179	Atorvastatin	CTD	8.017E-10
C007020	Chlorophyllin	CTD	4.567E-7
CID000005645	Ursodeoxycholic acid	Stitch	8.825E-7
D008070	Lipopolysaccharides	CTD	1.074E-6
D003907	Dexamethasone	CTD	1.204E-6
Primary sclerosing cholangitis			
CID000005645	Ursodeoxycholic acid	Stitch	9.387E-7
D001647	Bile acids and salts	CTD	1.703E-6
D002777	Cholestanols	CTD	1.902E-6
D014580	Ursodeoxycholic acid	CTD	7.575E-6
CID000000334	7alpha-hydroxy-4-cholesten-3-one	Stitch	8.391E-6

Predictions were done by ToppFun application (Pharmacome, Drug-Gene associations), based on data of CTD (Comparative Toxicogenomics Database) and Stitch (Search Tool for InTeractions of Chemicals).

Figure 1

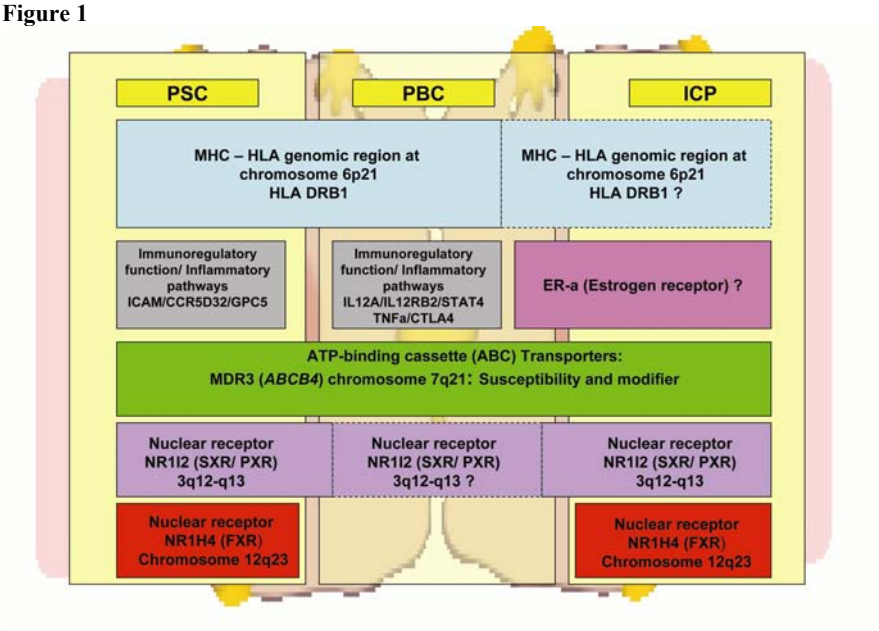
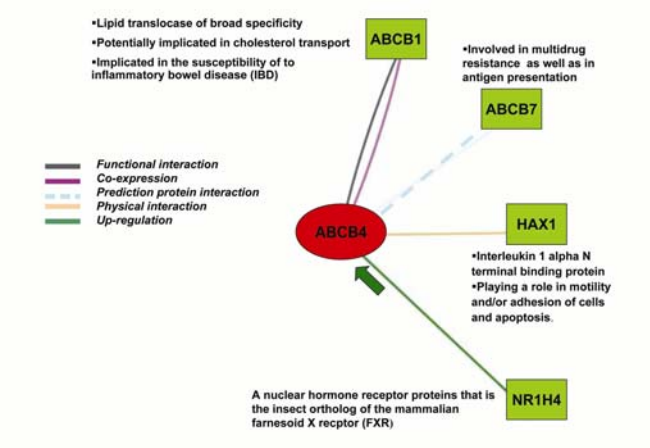


Figure 2



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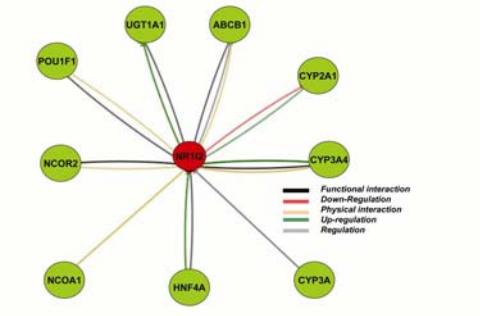

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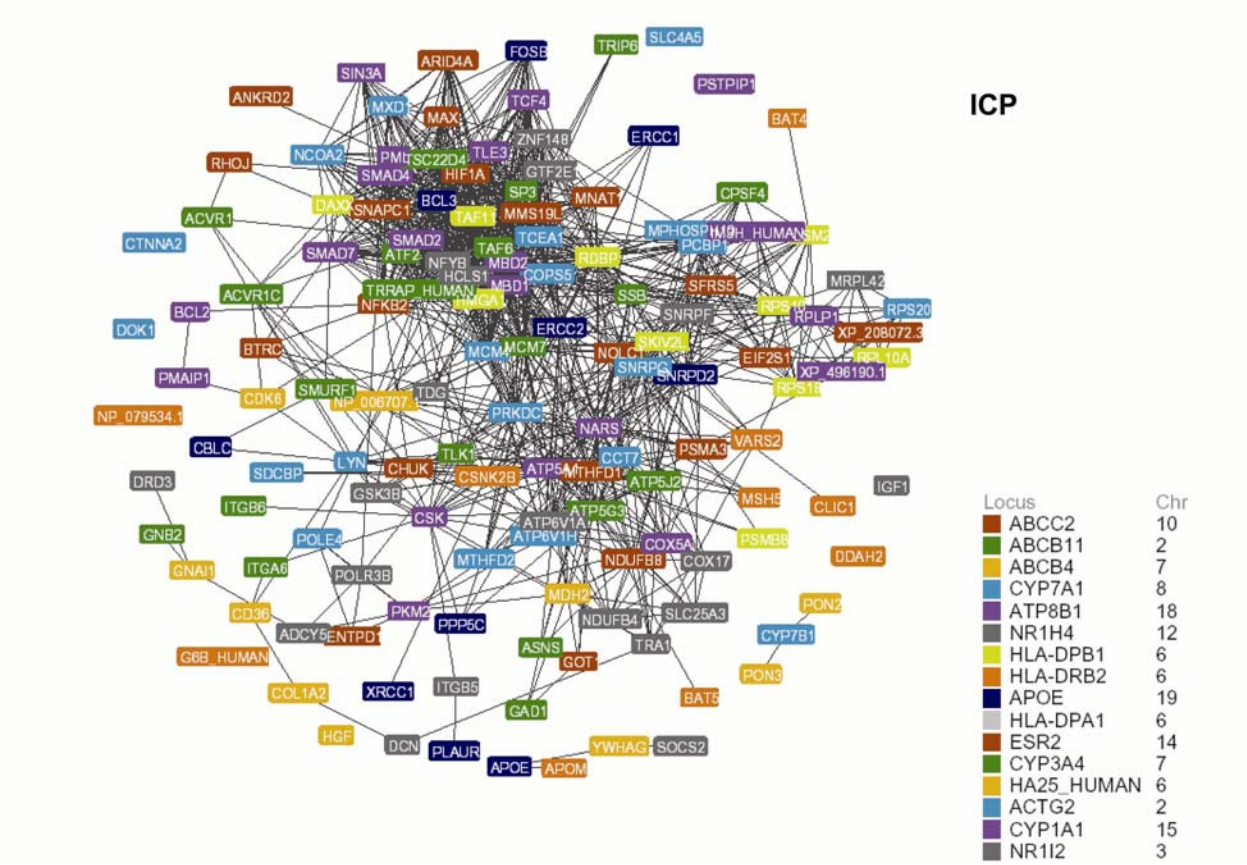
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ABCB4 7
CYP7A1 8
ATP8B1 18
NR1H4 12
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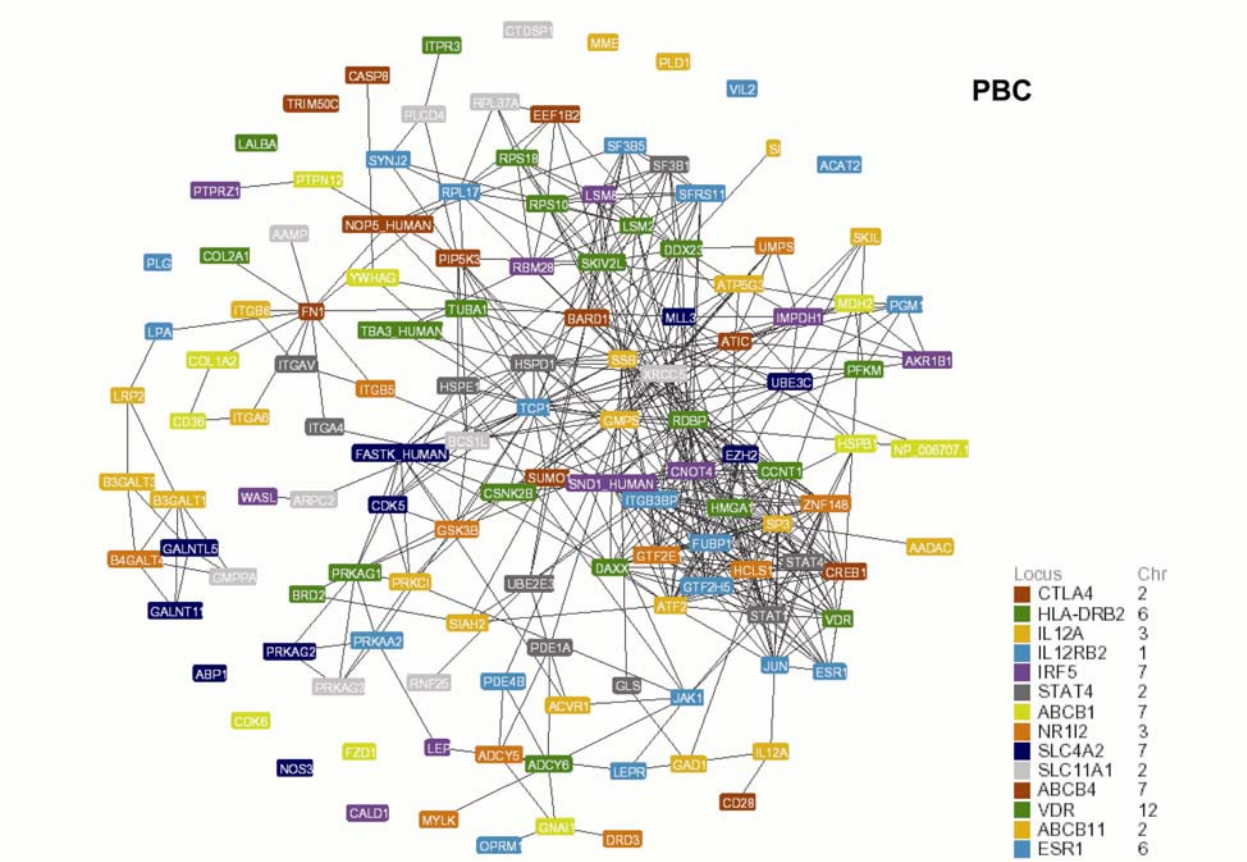
HLA-DRB2 6
APOE 19
HLA-DPA1 6
ESR2 14
CYP3A4 7
HA25_HUMAN 6
ACTG2 2
CYP1A1 15
NR1I2 3

Figure 5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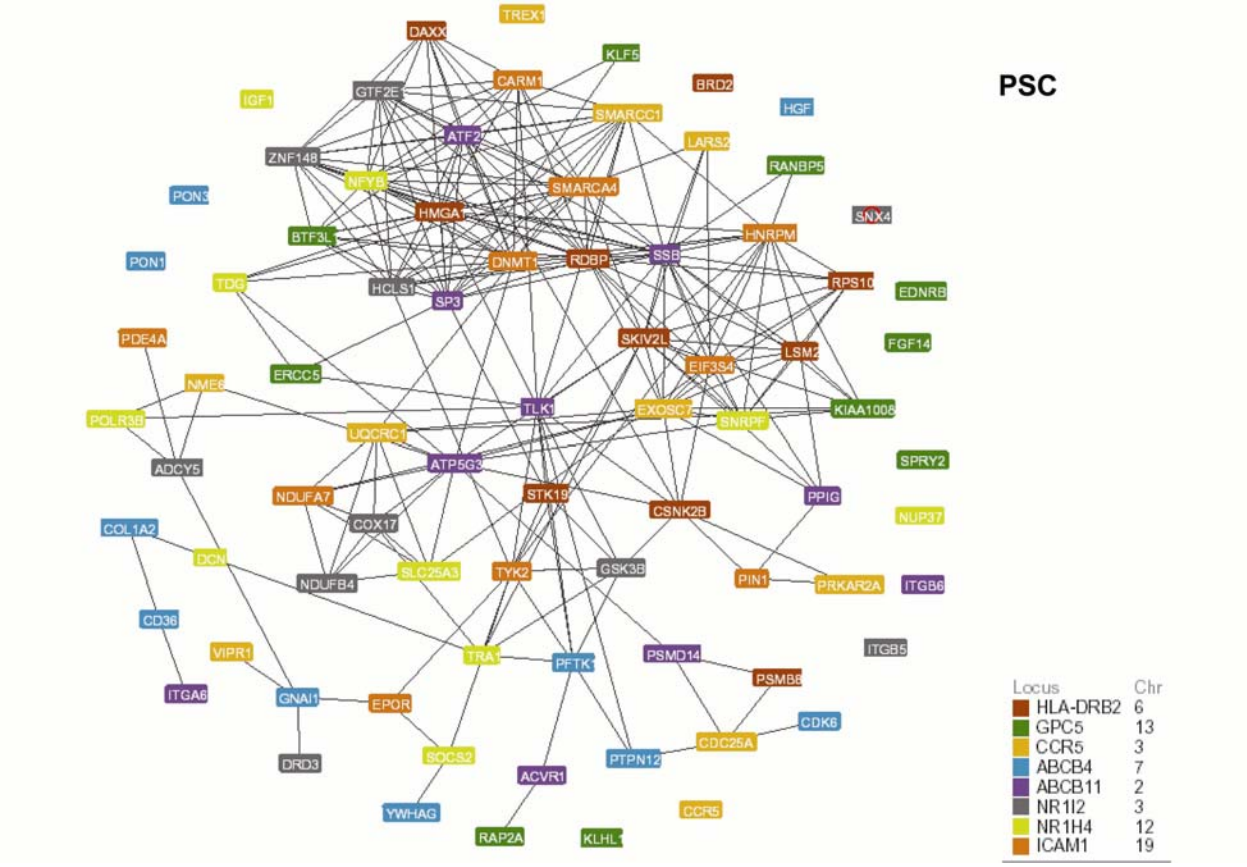
STAT4 2
ABCB1 7
NR1I2 3
SLC4A2 7
SLC11A1 2
ABCB4 7
VDR 12
ABCB11 2
ESR1 6

Figure 6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NFYB
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PON3
HMGA1
SNX4
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DNMT1
RDBP
SSB
TDG
HCLS1
SP3
RPS10
EDNRB
PDE4A
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KIAA1008
POLR3B
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ADCY5
ATP5G3
SPRY2
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PPIG
CSNK2B
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PIN1
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ITGB6
CD36
ITGB5
VIPR1
TRA1
PFTK1
PSMD14
PSMB8
ITGA6
GNAI1
EPOR
CDK6
CDC25A
DRD3
SOCS2
PTPN12
ACVR1
YWHAG
CCR5
RAP2A
KLHL1
Locus
Chr
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GPC5 13
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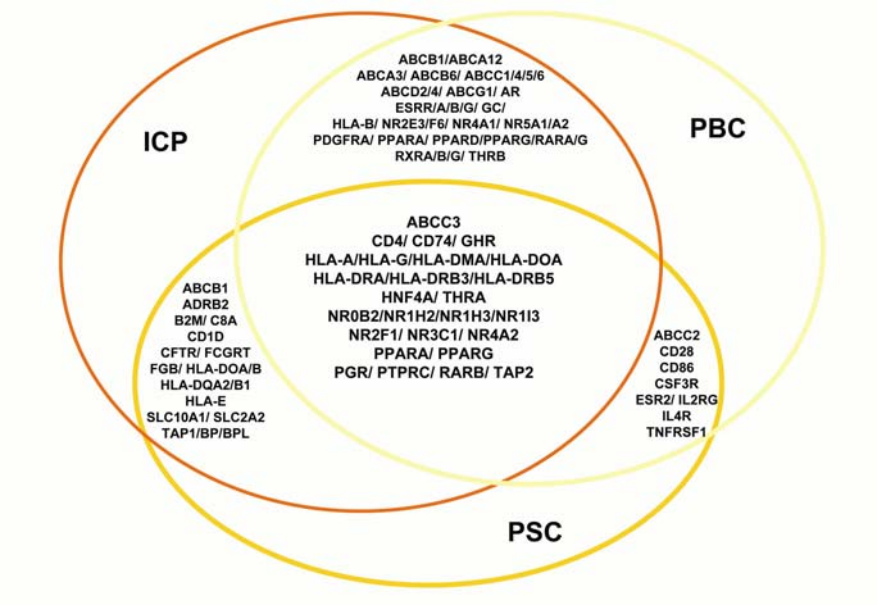
ABCB11 2
NR1I2 3
NR1H4 12
ICAM1 19

Figure 7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PSC
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ESRR/A/B/G/ GC/
HLA-B/ NR2E3/F6/ NR4A1/ NR5A1/A2
PDGFRA/ PPARA/ PPARD/PPARG/RARA/G
RXRA/B/G/ THRB
ABCC3
CD4/ CD74/ GHR
HLA-A/HLA-G/HLA-DMA/HLA-DOA
HLA-DRA/HLA-DRB3/HLA-DRB5
HNF4A/ THRA
NR0B2/NR1H2/NR1H3/NR1I3
NR2F1/ NR3C1/ NR4A2
PPARA/ PPARG
PGR/ PTPRC/ RARB/ TAP2
ABCB1
ADRB2
B2M/ C8A
CD1D
CFTR/ FCGRT
FGB/ HLA-DOA/B
HLA-DQA2/B1
HLA-E
SLC10A1/ SLC2A2
TAP1/BP/BPL
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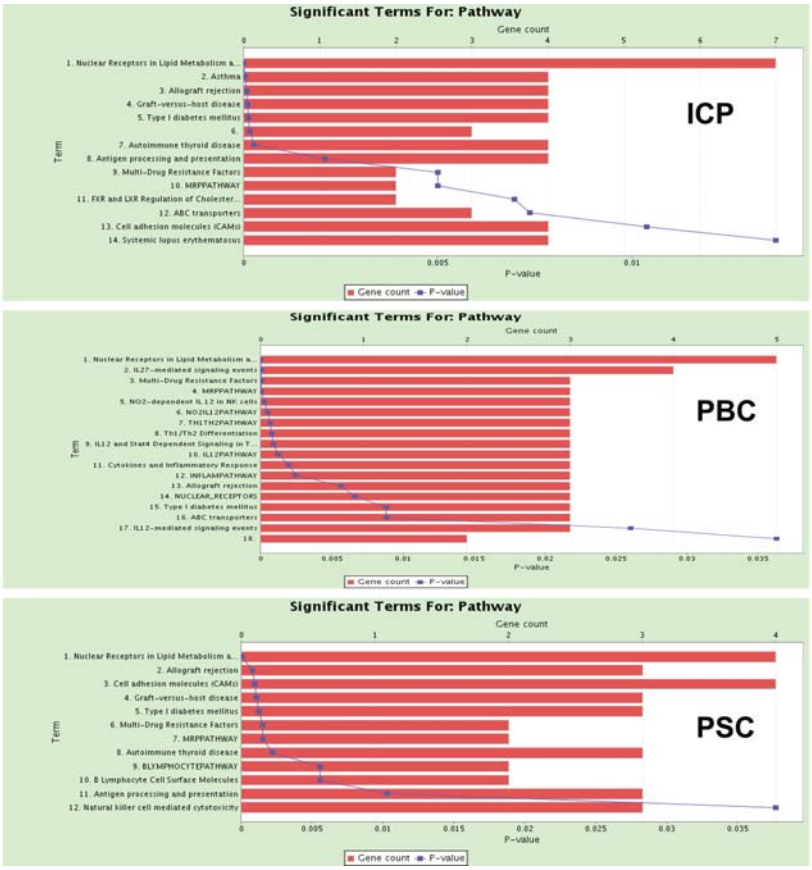
CD86
CSF3R
ESR2/ IL2RG
IL4R
TNFRSF1

Figure 8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3. Allograft rejection
4. Graft-versus-host disease
5. Type I diabetes mellitus
6.
7. Autoimmune thyroid disease
8. Antigen processing and presentation
9. Multi-Drug Resistance Factors
10. MRPPATHWAY
11. FXR and LXR Regulation of Cholester...
12. ABC transporters
13. Cell adhesion molecules (CAMs)
14. Systemic lupus erythematosus
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P-value
Gene count
P-value
ICP
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6. NO2IL12PATHWAY
7. TH1TH2PATHWAY
8. Th1/Th2 Differentiation
9. IL12 and Stat4 Dependent Signaling in T...
10. IL12PATHWAY
11. Cytokines and Inflammatory Response
12. INFLAMPATHWAY
13. Allograft rejection
14. NUCLEAR_RECEPTORS
15. Type I diabetes mellitus
16. ABC transporters
17. IL12-mediated signaling events
18.
Term
P-value
Gene count
P-value
PBC
Significant Terms For: Pathway
Gene count
1. Nuclear Receptors in Lipid Metabolism a...
2. Allograft rejection
3. Cell adhesion molecules (CAMs)
4. Graft-versus-host disease
5. Type I diabetes mellitus
6. Multi-Drug Resistance Factors
7. MRPPATHWAY
8. Autoimmune thyroid disease
9. BLYMPHOCYTEPATHWAY
10. B Lymphocyte Cell Surface Molecules
11. Antigen processing and presentation
12. Natural killer cell mediated cytotoxicity
Term
P-value
Gene count
P-value
PSC