Why Am I Yellow?
Answers from Shakespeare and HUGO

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Internal Medicine Grand Rounds
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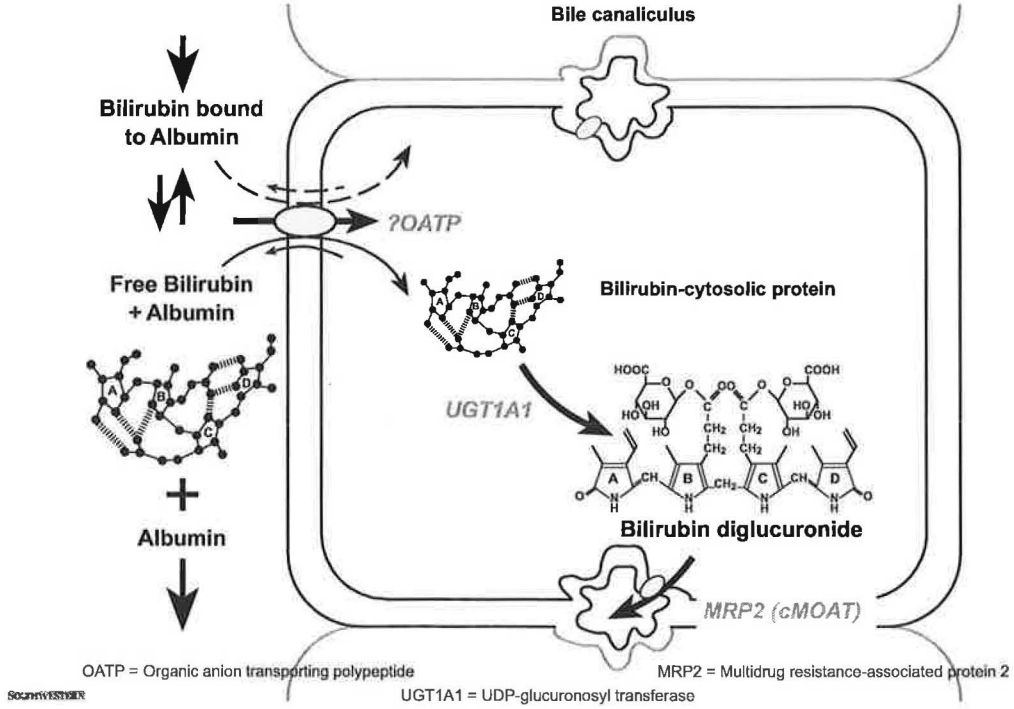

All the world's a stage,
And all the men and women merely players,
They have their exits and entrances,
And one man in his time plays many parts,
His acts being seven ages. At first the infant,
Mewling and puking in the nurse's arms.
Then, the whining schoolboy with his satchel
And shining morning face, creeping like snail
Unwillingly to school. And then the lover ,
Sighing like furnace, with a woeful ballad
Madew to his mistress' eyebrow. Then a soldier,
Full of strange oaths, and bearded like the pard,
Jealous in honour, sudden, and quick in quarrel,
Seeking the bubble reputation
Even in the cannon's mouth. And then, the justice
In fair round belly, with good capon lin'd,
With eyes severe, and beard of formal cut,
Full of wise saws, and modern instances,
And so he plays his part. The sixth age shifts
Into the lean and slipper'd pantaloon,
With spectacles on nose, and pouch on side,
His youthful hose well sav'd, a world too wide,
Fir his shrunk shank, and his big manly voice,
Turning again towards childish treble, pipes
And whistles in his sound. Last scene of all,
That ends this strange eventful history,
Is second childishness and mere oblivion,
Sans teeth, sans eyes, sans taste, sans everything,

From: As You Like It. Shakespeare, Wm.

This is to acknowledge that Dr. Jennifer Cuthbert has not disclosed any financial interests or other relationships with commercial concerns related directly to this program. Dr. Cuthbert will not be discussing off-label uses in her presentation.

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I greatly appreciate the advice and information that I received from Laura N. Bull, Ph. D. University of California, San Francisco and Alexander S. Knisely, M.D., Institute of Liver Studies, King's College Hospital, Denmark Hill, London, U.K.

GOALS

To provide some of the answers to the question "Why Am I Yellow?"
To provide some of the tools needed to answer this question in most patients.

LEARNING OBJECTIVES

1. Define hyperbilirubinemia and jaundice
2. Identify pathway for normal bilirubin metabolism
3. Identify three major anatomic categories for etiology of jaundice
4. Distinguish between jaundice and cholestasis
5. Recognize patterns of liver tests associated with jaundice from different etiologies
6. Identify inherited causes of jaundice
7. Understand importance of jaundice in chronic, non-cholestatic liver disease

INTRODUCTION

"Sorry for this email "consult" but, a 66 yo Mexican male was admitted to our PMH service with fever and was diagnosed with pyelonephritis. Of note his total bili was 10.5 and direct 5.5 with otherwise completely normal liver numbers-including normal INR and albumin. The only old numbers we have from June obtained from urology clinic show small bili in his urine (which was large this admission). He is icteric of course but the patient doesn't recognize any change to his appearance or urine color. He has received no other medical care and lived most of his life in Mexico. We are trying to get information from other family members. He has responded nicely to therapy for his infection and has no complaints. My housestaff is suppose to discuss this with the liver fellow and arrange follow-up but could this be anything other than a type II Crigler-Najjar syndrome?"

The answer is "Yes" there are other causes of hyperbilirubinemia with neither other abnormalities of liver function nor evidence of hepatic inflammation. Hyperbilirubinemia = a bilirubin level above normal. In isolated hyperbilirubinemia, by definition, only the bilirubin is abnormal. This distinction is useful in a systematic approach to the diagnosis of the cause of jaundice. Jaundice is the clinical appreciation of hyperbilirubinemia. Jaundice and icterus are synonymous, icterus does not refer to jaundice of the patient's eyes.

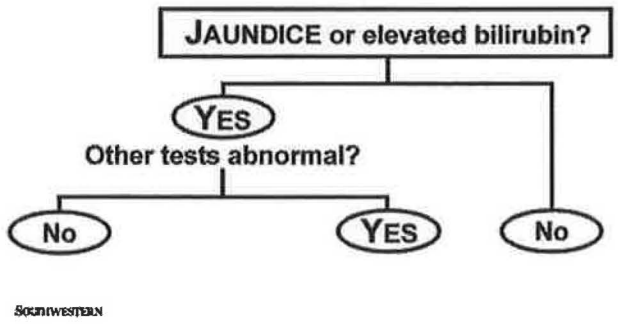


Figure 1: A Systematic Approach to Abnormal Liver Tests - Diagnosing the Cause of Jaundice

BIOCHEMISTRY AND PHYSIOLOGY OF BILIRUBIN AND BILE

The liver has both exocrine and endocrine functions. Its exocrine function is that of the formation and excretion of bile. The synthesis and secretion of virtually all the circulating plasma proteins (with the exception of the immunoglobulins) can be considered its "endocrine" function. In addition, the liver plays an important role in the normal metabolism of carbohydrates, lipids and proteins and is the major storage compartment for vitamins A and B12.

Bilirubin is the end-product of heme breakdown. When red blood cells become senescent and are phagocytosed, their content of hemoglobin is degraded by the heme oxygenase system of the mononuclear phagocytes. The iron

and the amino acids from the globin chains are recycled whereas the heme moiety is converted to bilirubin IXα by biliverdin reductase which opens the α link between the A and D pyrrole rings. The heme within other hemoproteins, e.g. cytochromes, catalase in the liver, kidney etc., can be similarly metabolized. Heme from red blood cells constitutes ~70% of normal bilirubin production, the remainder being supplied by the breakdown of other hemoproteins. Bilirubin IXα is non-polar and lipid-soluble, circulating largely bound to albumin before being taken up by the liver.

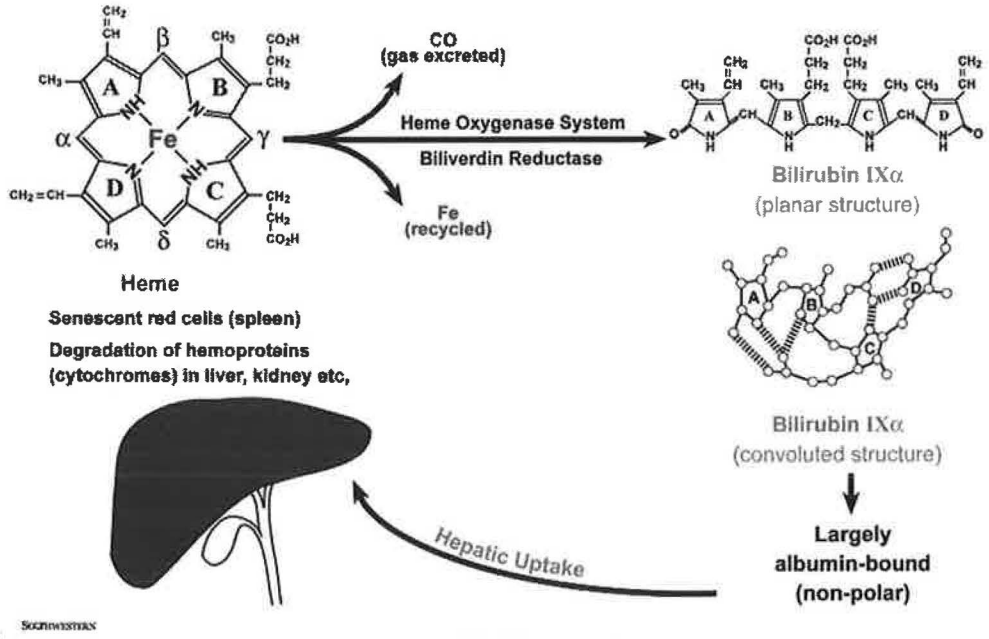


Figure 2: Bilirubin Formation

The mechanism of hepatic uptake of bilirubin into the cell, across the basolateral membrane that borders the hepatic sinusoid, is controversial. Data generally indicate that a specific carrier is involved. Some investigators implicate OATP2, an organic anion transporting polypeptide. However, others consider that there may only be passive diffusion with rapid binding and metabolism resulting in a concentration gradient.

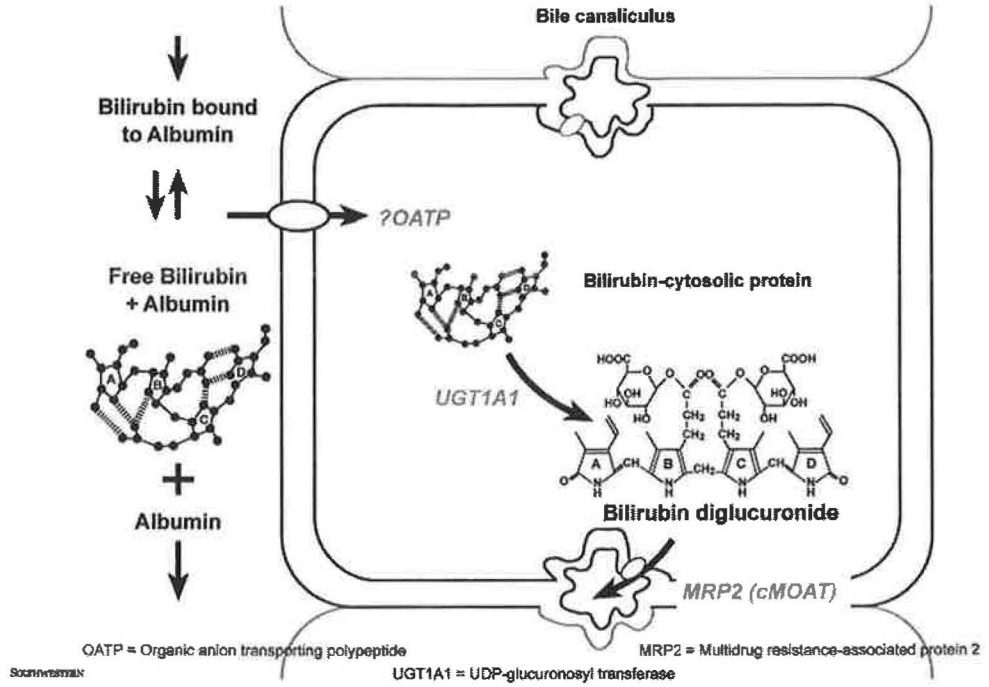


Figure 3: Metabolism of Bilirubin

Once in the hepatic parenchymal cells, bilirubin forms complexes with cytosolic chaperone proteins, the glutathione-S-transferases, also termed ligandins or Y-proteins. The enzyme uridine diphosphate glucuronosyl transferase (UDP-glycosyl transferase 1 family, UGT1A1) catalyzes the sequential addition of two glucuronide groups forming conjugated bilirubin. UGT1A1 is one member of the UDP-glycosyl transferase family 1. All UGT1 mRNA species share the terminal 4 exons, numbers 2-5. Each has a unique first exon with an accompanying promoter specific for exon 1. Only UGT1A1 is specific for bilirubin as a substrate. Its TATA box is $A(TA)_6ATAA$ In Gilbert syndrome, the commonest cause of unconjugated hyperbilirubinemia, the promoter has an extra TA. Conjugated bilirubin, = bilirubin diglucuronide, is a water-soluble, polar compound.

Excretion of conjugated bilirubin, i.e. the diglucuronide form, occurs across the apical membrane of the hepatocyte into the biliary canaliculus via the ATP-dependent transporter MRP2, = **M**ultidrug **R**esistance-associated **P**rotein 2, encoded by the gene ABCC2, the C2 member of the ABC [ATP-binding cassette] transporter family. MRP2 was also termed the canalicular **M**ultispecific **O**rganic **A**nion **T**ransporter, cMOAT. From the hepatocyte canaliculus, bilirubin in bile flows through the canals of Hering into increasingly larger bile ducts and the intestine.

SUMMARY: Bilirubin Metabolism			
Process	**Gene**	**Gene Nomenclature**	**Product**
Uptake	? SLC21A6 **604843*	Solute carrier family 21 member 6	Organic anion transporting polypeptide2, OATP2, = OATP-C
Glucuronidation	UGT1A1 *#191740*	UDP-glycosyl transferase 1 family, member A1	UDP-glycosyltransferase 1 = UDP-glucuronosyl transferase
Excretion	ABCC2 **601107*	ATP-binding cassette family, C2 member	Multidrug resistance-associated protein 2 MRP2
Storage ?	Unknown		Mutated in Rotor syndrome (see below)
The numbers in italics refer to OMIM Online Mendelian Inheritance in Man entries * prefix = gene of known sequence # prefix = descriptive entry, does not represent a unique locus			

The normal serum bilirubin is ≤1.3 mg/dl with conjugated bilirubin i.e. bilirubin diglucuronide constituting <1% of the total. Direct bilirubin levels reported for normal people using routine tests are not the equivalent of the true level of conjugated bilirubin. The methodology required to detect normal conjugated bilirubin is not available in standard laboratories.

The upper limit of the range for normal bilirubin levels is lower in women than in men. The precise level of normal serum bilirubin is determined by the input into the circulation = heme breakdown and the output from the circulation into the liver = hepatic uptake.

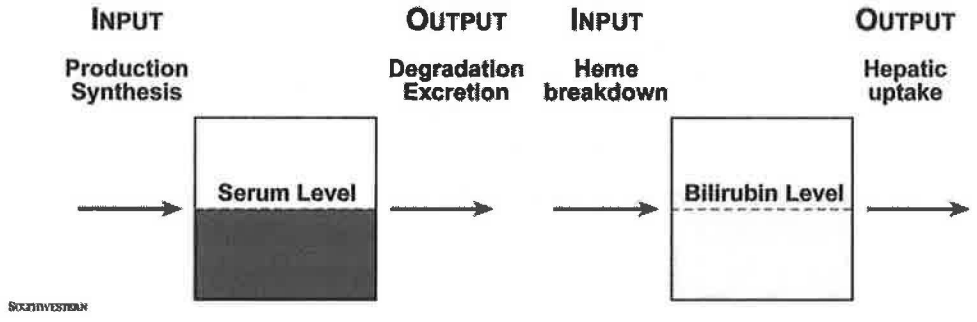


Figure 4: Control of Normal Serum Bilirubin

After excretion into the biliary canaliculus, bilirubin becomes one of the components of bile and thus part of the liver's exocrine function. The major components of bile include bilirubin, bile acids and biliary lipids. The specific transporters involved in the secretion of individual components across the hepatocyte apical (canalicular) membrane are shown below.

Normal Hepatic Exocrine Physiology		
Function	**Gene**	**Nomenclature and Transporter Product(s)**
Excretion of bile acids for digestion of fat and fat-soluble vitamins	ABCB11 *603201	ATP-binding cassette subfamily B member 11 = BSEP (bile salt export pump)
Excretion of end-product of heme breakdown (bilirubin)	ABCC2 *601107	ATP-binding cassette subfamily C member 2 = MRP2 (multidrug resistance-associated protein 2)
Transformation and excretion of drugs & toxins	ABCB1 *171050	ATP-binding cassette subfamily B member 1 = MDR1 (multidrug resistance type 1) Also MDR3 and MRP2
Excretion of biliary cholesterol	ABCG5 *605459 ABCG8 *605460	ATP-binding cassette subfamily G member 5 (heterodimerizes with ATPG8) ATP-binding cassette subfamily G member 8 (heterodimerizes with ATPG5)
Excretion of biliary phospholipid	ABCB4 *17160	ATP-binding cassette subfamily B member 4 = MDR3 (multidrug resistance type 3)
? Aminophospholipid translocator	ATP8B1 *602397	ATPase class 1, type 8B, member 1 = FIC1 (familial intrahepatic cholestasis type 1)

The numbers in italics refer to OMIM Online Mendelian Inheritance in Man entries
* prefix = gene of known sequence

The contents of bile include bile acids, biliary lipids, assorted proteins plus bilirubin in a water-based fluid containing electrolytes, predominantly Na^+, K^+, Ca^{++}, Cl^- and $HCO3^-$.

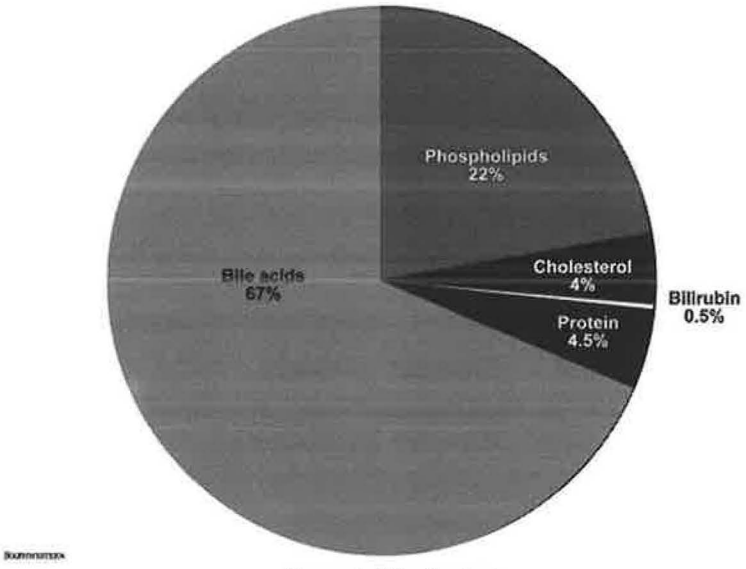


Figure 5: Bile Contents

The primary bile acids, cholic (3α, 7α, 12α tri-hydroxy) and chenodeoxycholic (3α, 7α di-hydroxy) are synthesized from cholesterol in the liver. Conversion of cholesterol to bile acids is the only major way of removing cholesterol from the body. The hydroxylation steps provide polar groups for interaction with water molecules. Secondary bile acids, deoxycholic (3α, 12α di-hydroxy), lithocholic (3α mono-hydroxy) and

ursodeoxycholic (3α, 7β di-hydroxy) are formed in the intestine by the action of the normal bacterial flora. The bile acids are conjugated to either glycine or taurine in the liver and excreted as the conjugates into bile.

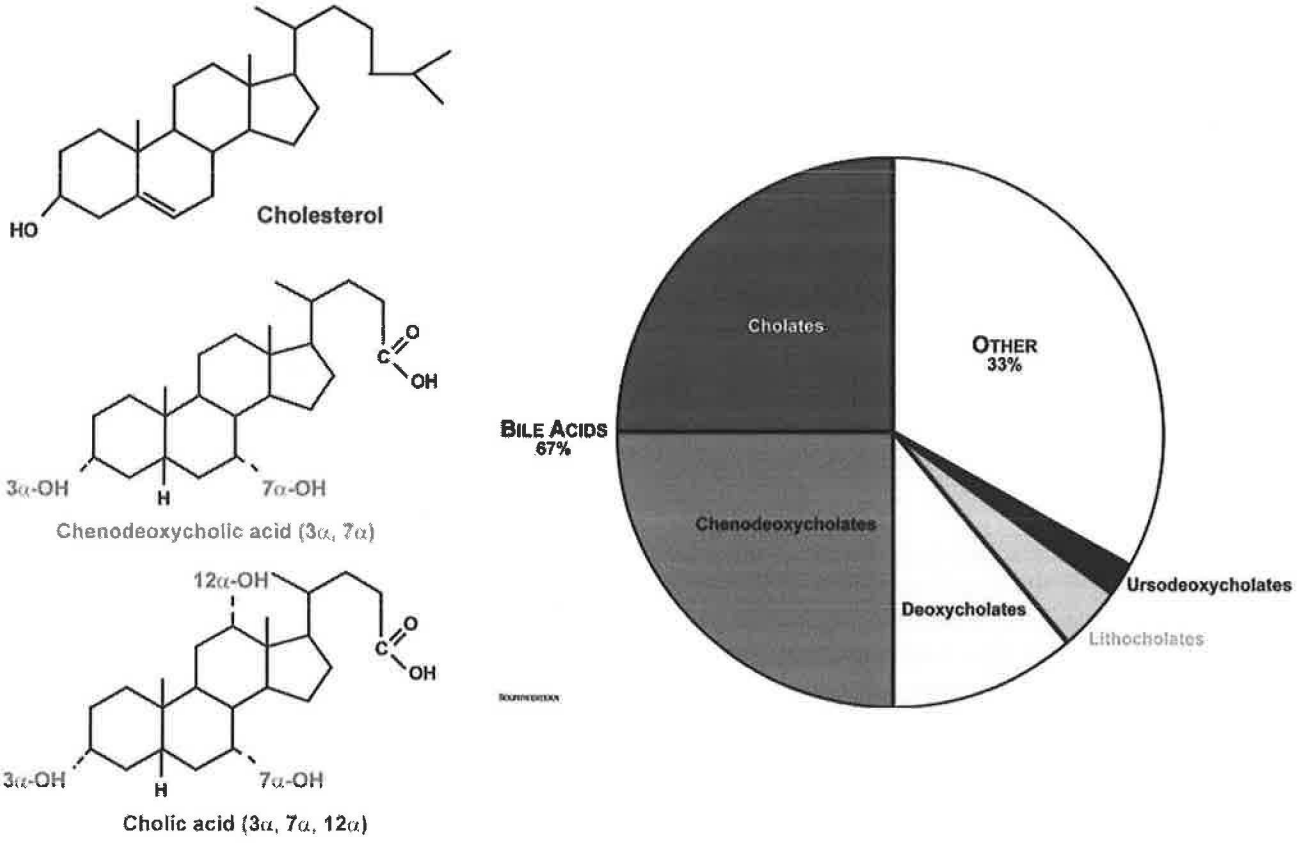


Figure 6: Bile Acid Component of Bile

Synthesis and fecal excretion of bile acids each day totals 0.2-0.6 g when there is a balanced state. However, there is secretion (excretion) of 12-36 g/d from the liver because the bile acid pool of ~3 g undergoes an enterohepatic circulation, cycling 4-12 times per day.

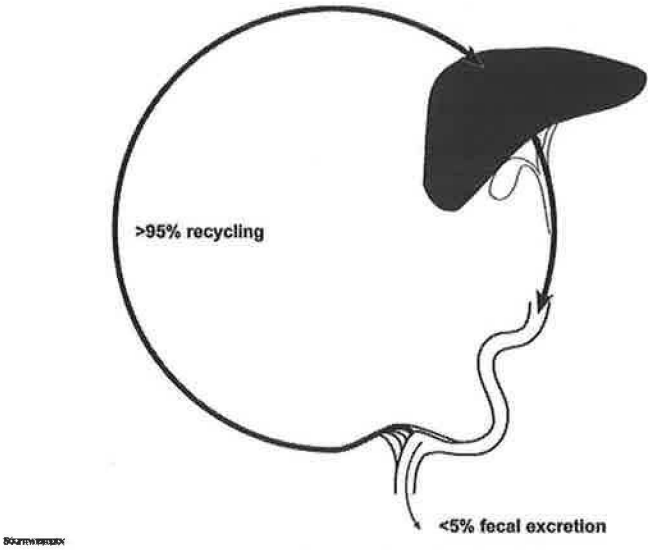


Figure 8: Enterohepatic Circulation

A specific transporter in the terminal ileum (ISBT, **I**leal **S**odium **B**ile acid co**T**ransporter) returns 95% of intestinal bile acids to the portal circulation. Another specific transporter (NTCP, sodium [**N**a]-**T**aurocholate **C**otransporting **P**olypeptide) is involved in hepatic uptake of bile acids for re-excretion into bile. Only the 0.2-0.6

g/d that escape recycling, under normal circumstances, needs to be replaced by newly synthesized bile acids. The major function of bile acids is to aid in the secretion of biliary lipids and the digestion of fats and fat-soluble vitamins by forming micelles. The bile acids are amphipathic molecules, their cholesterol backbone being non-polar and lipid-soluble while their hydroxyl groups provide a polar, water-soluble surface. By surrounding the lipid interfaces of biliary cholesterol and phospholipids, bile acids form micelles that can then be used in the digestion and absorption of dietary lipids.

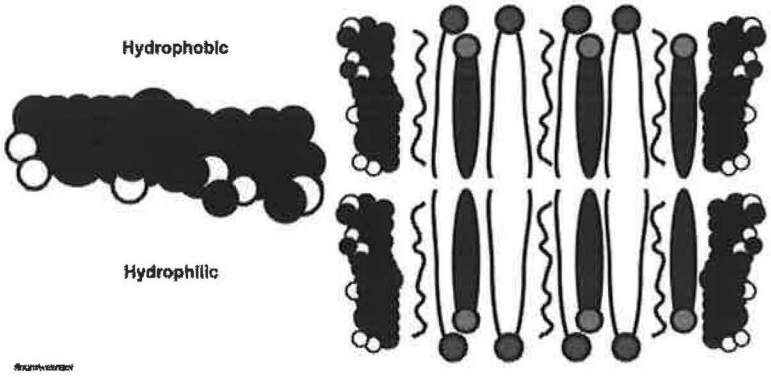


Figure 9: Function of Bile Acids

Phospholipids, mainly phosphatidyl choline (lecithin) and cholesterol constitute the biliary lipids. Phospholipids are transported across the apical membrane of the hepatocyte into the biliary canaliculus by MDR3. MDR3 is also involved in the transport of drugs and xenobiotics and was first identified because of its association with the **M**ulti **D**rug **R**esistance phenotype in cancer therapy. Studies in knockout mice have clearly demonstrated that targeted mutation of the murine equivalent of the gene (*mdr2*) totally blocks phospholipid transport into bile.

Cholesterol is transported across the canalicular membrane of the hepatocyte by the heterodimer ABCG5 / ABCG8. These are ATP-binding cassette (ABC) family members that are also involved in transporting cholesterol across the intestine. Dr. Helen Hobbs reviewed the classification and function of the ABC transporters in January 2003.

PATHOLOGY OF HYPERBILIRUBINEMIA, JAUNDICE AND CHOLESTASIS

Hyperbilirubinemia, an increase in the circulating level of bilirubin, can be an isolated abnormality as in the patient described above or can occur in association with other findings suggesting liver and/or biliary tract disorders. An isolated increase in bilirubin alone can be the result of hemolysis, a *pre-hepatic* disorder, or defects in hepatic bilirubin metabolism, *hepatic* disorders.

Hemolysis = Pre-hepatic:

1. Increased bilirubin production i.e. excessive red cell destruction = hemolysis e.g. from drug reaction, red cell enzyme deficiency etc.

Bilirubin Metabolism Defect = Hepatic:

2. Decreased hepatic uptake of unconjugated bilirubin

3. Defective metabolism = decreased conjugation with glucuronide

4. Impaired excretion into the biliary canaliculus

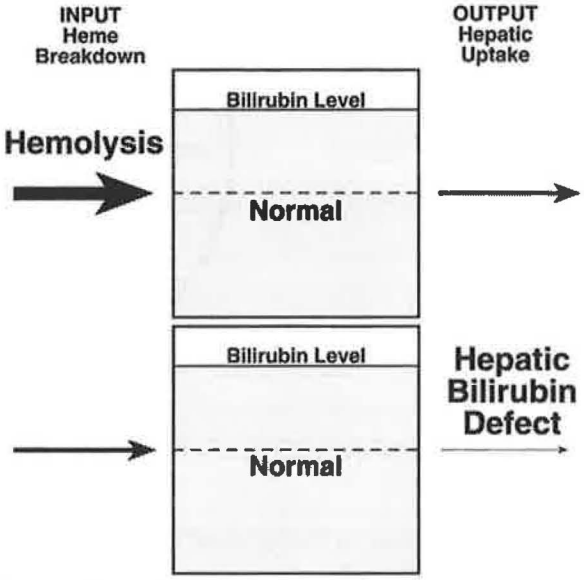


Figure 10: Isolated Elevation of Serum Bilirubin Levels

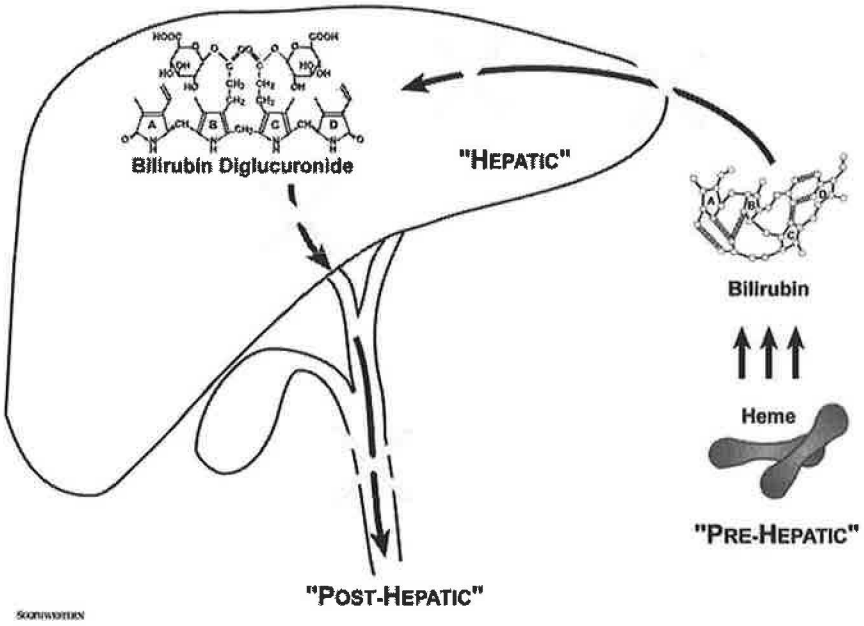


Figure 11: Anatomic Pathology of Hyperbilirubinemia

Hepatic and *post-hepatic* disorders that lead to elevations in both the unconjugated fraction and the conjugated fraction of bilirubin are the commonest causes of hyperbilirubinemia. In these diseases, there is almost always another abnormality, either in hepatic enzyme levels (aminotransferases, alkaline phosphatase) or estimations of hepatic function (albumin, prothrombin time) or both. Isolated hyperbilirubinemia, with elevations of both unconjugated and conjugated bilirubin fractions, is uncommon to rare depending on the population under study.

The increased circulating bilirubin can be observed as jaundice or icterus of the skin or eyes when the level exceeds 2-3 mg/dl. Bilirubin binds to the elastic fibers of the subepithelial lamina propria, which is the innermost layer of the conjunctiva, and the contiguous episclera, not to the collagenous sclera proper.

Although icterus and jaundice are synonymous, jaundice and cholestasis are not. Jaundice results from abnormalities in the production and excretion of bilirubin. Cholestasis results from any impairment in the production and excretion of bile. Bilirubin is only one of the components of bile.

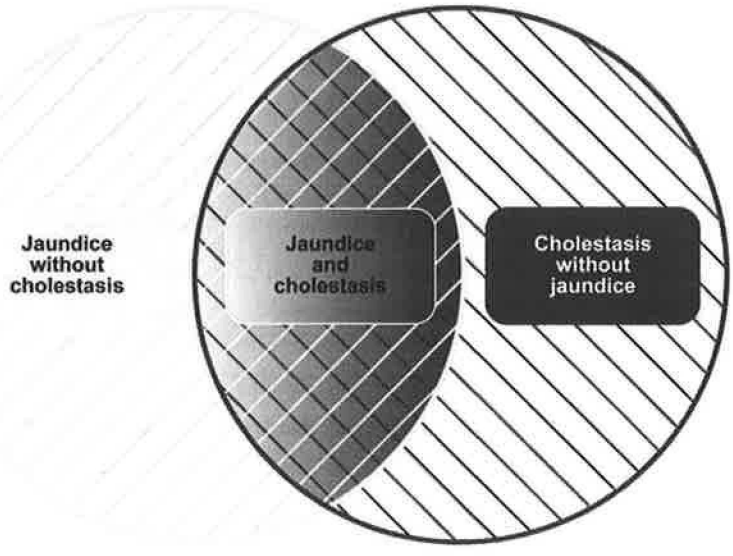


Figure 12: Relationship Between Jaundice and Cholestasis

Conceptually, jaundice and cholestasis are separable entities. Thus, for example, jaundice from hemolysis occurs in the complete absence of any cholestasis or other evidence of liver disease. At the other end of the spectrum, obstruction can be associated with cholestasis and a variety of accompanying symptoms and signs in the absence of jaundice, for example IF the obstruction to bile flow is incomplete or is patchy and only effects part of the liver. More commonly, however, jaundice and cholestasis are encountered together when the normal "exocrine" function of the liver, the formation and excretion of bile and its contents including bilirubin, is affected by a diffuse acute or advanced chronic disease process.

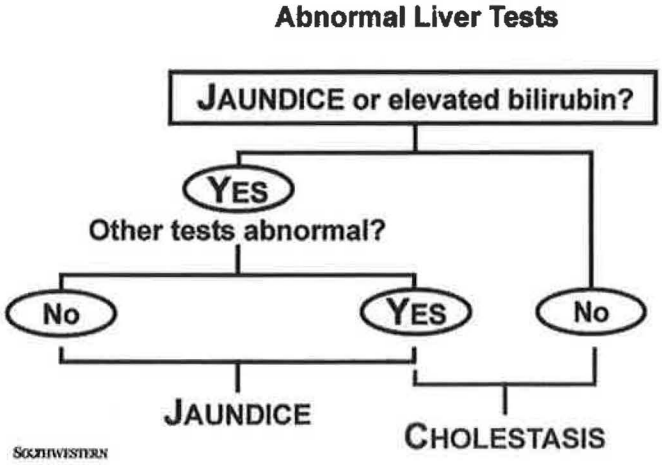


Figure 13: Systematic Approach to the Diagnosis of Jaundice and Cholestasis

Bile flow can be interrupted at the level of the hepatocyte by intra-cellular disorders that interfere with secretion into the biliary canaliculus. Genetic defects of the canalicular transporters are rare however drugs and hormones can interfere with their function. Alternatively, cholestasis may originate from an intra-hepatic disorder that physically obstructs or destroys bile ducts, such as infiltration of portal tracts with granulomas or portal fibrosis or immune-mediated destruction. Finally, an extra-hepatic process with resultant mechanical obstruction to bile flow, such as benign strictures or tumors of the bile duct or pancreas may be the cause of interrupted bile flow and cholestasis.

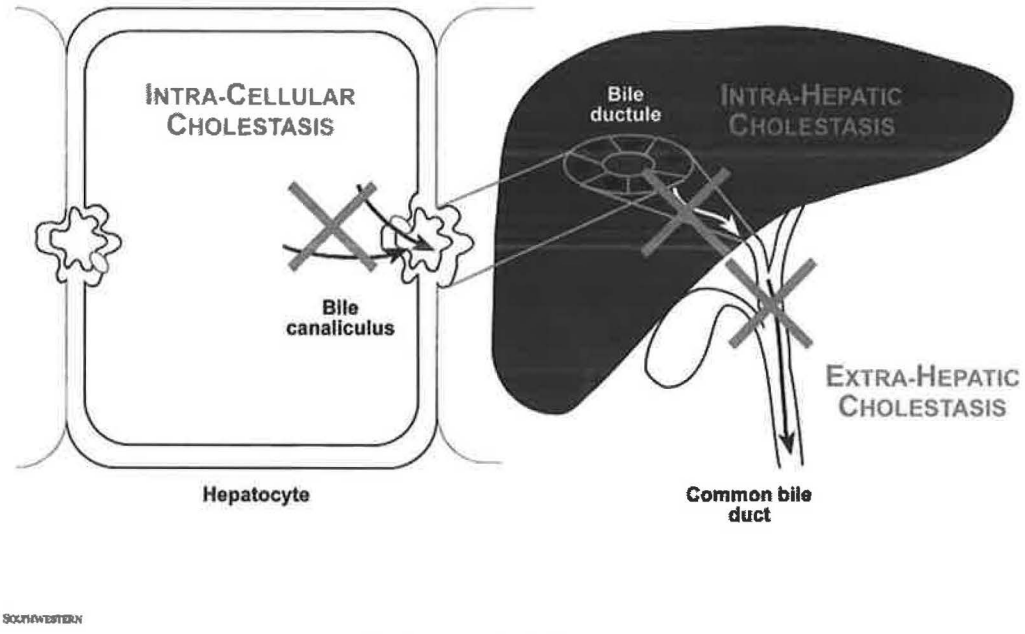


Figure 13: Anatomic Pathology of Cholestasis

GENETICS OF HYPERBILIRUBINEMIA, JAUNDICE AND CHOLESTASIS

Hepatic uptake of bilirubin:
There are no known genetic diseases with abnormalities in hepatic uptake of bilirubin. The difficulty in isolating a single transport protein for this function may be related to the presence of a number of transporters with overlapping specificities and transport kinetics. Candidates include members of the organic anion transporters in solute carrier family 21, OATP2 from the SLC21A6 gene and OATP8 from the SLC21A8 gene. Both proteins transport bromosulfophthalein (BSP) a manufactured testing substance that is transported into hepatocytes and excreted into bile.

Uptake of drugs and other xenobiotics by the liver is often necessary for activation, metabolism or excretion. A variety of transporter proteins and polypeptides on the basolateral i.e. sinusoidal membrane can accomplish uptake of drugs into the hepatocyte. Inhibition of various transporters by drug interactions can be part of a hepatotoxicity reaction. Polymorphisms in hepatic transporter genes may explain drug sensitivities and idiosyncratic reactions.

Hepatic secretion of conjugated bilirubin into the sinusoid:
Secretory organic anion transporters on the basolateral membrane of hepatocytes include MRP1, MRP3 and MRP6. All are members of the ABCC family of transporters. Experimental sepsis models of cholestasis induce MRP1 and MRP6 expression whereas obstruction induces MRP3. MRP1 and MRP3 may pump bilirubin glucuronides into the circulation in disease.

Hepatic conjugation of bilirubin producing bilirubin diglucuronide:
Conjugation of bilirubin is catalyzed by UDP-glucuronosyl transferase encoded by UGT1A1. Gilbert syndrome has very mild conjugation defects and is common, being detected in up to 9% of the population. Severe defects in conjugation activity are rare.

The inheritance of Gilbert syndrome is autosomal recessive. Data indicate that the decrease in conjugation activity results from a mutation in the promoter region of the gene encoding UDP-glucuronosyl transferase, with $A(TA)_7TAA$ instead of $A(TA)_6TAA$. The variant allele is common (30%) with 9% homozygosity. The promoter mutation is also present in the absence of phenotypic Gilbert syndrome, suggesting that a second factor may be involved. Gilbert syndrome is diagnosed more frequently in males than females, perhaps reflecting the lower normal levels of bilirubin in women and consequently a more severe defect required to exceed the normal level (which is not sex-specific) in a general population. The co-existence of mild conjugation defects with increased hemolysis and with abnormalities of bilirubin uptake may reflect the commonness of Gilbert syndrome and increasing likelihood of detection when a second disorder is present.

The UGT1A family of glucuronidation enzymes shares all but the first exon. Substrate specificity is determined by this first exon and expression levels are dependent on the exon-1 linked promoter. Alterations of drug metabolism in Gilbert syndrome may be the result of decreased hepatic uptake or a polymorphism in another glucuronidation enzyme. The association with Gilbert syndrome may reflect inheritance of two separate but linked polymorphisms in the UGT1A locus.

More severe conjugation defects are rare. In Crigler-Najjar type II, UDP-glucuronosyl transferase activity is low but can be induced by treatment with phenobarbital that induces the activity of numerous hepatic enzymes. The defect in Crigler-Najjar type I is more extensive and increased activity of the enzyme cannot be induced by phenobarbital treatment. These patients are at risk for kernicterus in adolescence and liver transplantation should be considered for management. Both type I and type II variants of Crigler-Najjar are autosomal recessive disorders. Conjugation defects are isolated abnormalities in bilirubin metabolism rather than more generalized hepatic or biliary disorders and therefore the patients lack any symptoms or signs of cholestasis. The Gunn rat is an animal model of Crigler-Najjar type I.

Hepatic excretion of conjugated bilirubin:
Isolated defects in the excretion of bilirubin from the hepatocyte across the apical membrane into the biliary canaliculus are rare. MRP2 [cMOAT] is the major transporter for conjugated bilirubin and mutations in both

genes results in Dubin-Johnson syndrome. It is inherited as an autosomal recessive disorder. Patients may be true homozygotes particularly if there is a history of consanguinity. Many patients are compound heterozygotes. The TR rat, the Eisai hyperbilirubin rat and the radixin knockout mouse are animal models of Dubin-Johnson syndrome. Biliary membrane localization of MRP2 may require radixin.

Dubin-Johnson syndrome is a completely benign form of isolated hyperbilirubinemia. The level of circulating unconjugated and conjugated bilirubin may further increase in the presence of sepsis or other disorders that independently lead to jaundice. MRP3, expressed on the basolateral surface of the hepatocyte can excrete bilirubin glucuronides into the sinusoid, thereby accounting for the elevated conjugated bilirubin in the absence of hepatocellular injury. The multispecific nature of the transporter is illustrated by the finding of alterations in urinary coproporphyrins and in the accumulation of a black pigment (NOT bilirubin) in the liver. Of note, the actual concentration of bilirubin in bile is normal. Other canalicular transporters can thus function when the concentration of bilirubin glucuronides increases in hepatocytes.

Expression of MRP2 is decreased in experimental models of sepsis and biliary obstruction. In contrast, MRP and MRP3 are induced by sepsis and biliary obstruction respectively. Both MRP1 and MRP3 are expressed on the basolateral membrane. Consequently, bilirubin glucuronides and other organic anions can be excreted into the circulation during obstruction and sepsis in the absence of hepatocellular injury.

Rotor syndrome is very rare. It is similar to Dubin-Johnson syndrome in phenotype. Both demonstrate benign unconjugated and conjugated hyperbilirubinemia and alterations in urinary coproporphyrin excretion patterns. However, no pigment accumulates in the liver of Rotor syndrome patients. Studies with BSP suggest that there may be a defect in storage of bilirubin rather than in excretion. The responsible gene is unknown.

SUMMARY: Genetics of Bilirubin Metabolism

Process	Gene	Product	Genetic Disorder(s)
Uptake	? SLC21A6 **604843*	Organic anion transporting polypeptide2	None known
Glucuronidation	UGT1A1 *#191740*	UDP-glycosyltransferase family 1 = UDP-glucuronosyl transferase	Gilbert syndrome *#143500* Crigler-Najjar I *#218800* Crigler-Najjar II *#606785*
Excretion	ABCC2 **601107*	Multidrug resistance-associated protein 2	Dubin-Johnson syndrome *#237500*
	Unknown		Rotor syndrome *%237450*

The numbers in italics refer to OMIM Online Mendelian Inheritance in Man entries
* = gene of known sequence
# = descriptive entry, usually of a phenotype, does not represent a unique locus
% = a confirmed mendelian phenotype or phenotypic locus for which the underlying molecular basis is not known

Hepatic excretion of bile acids:
Genetic defects leading to cholestasis and jaundice are well recognized in pediatrics. Many of the syndromes demonstrate autosomal recessive inheritance with multiple different mutations implicated. Before the mapping and cloning of the responsible genes, different phenotypes were delineated. At least three different syndromes of progressive familial intra-hepatic cholestasis (PFIC) were identified, based on the natural history and the presence

or absence of an elevated γ-glutamyl transpeptidase (GGT). GGT is NOT elevated in types 1 and 2, but is elevated in type 3. The natural history of type 1 is initially intermittent whereas type 2 is inexorably progressive. In addition, the bile is characteristically different on microscopic analysis. Identification of the responsible genes has permitted more precise descriptions of these diseases.

PFIC type 1 and PFIC type 2 are similar in that hepatic excretion of bile acids is defective. In PFIC type 2, it is clearly the result of an absence of the bile salt export protein and consequently a primary effect. Whether the failure to excrete bile acids is a primary or secondary event in PFIC type1 is unclear.

FIC1 familial intra-hepatic cholestasis type 1 (encodes a *P-type ATPase;* ? aminophospholipid translocase)*:*
Byler disease was first described in individual members of an extended Amish kindred. All affected children were descendants of Jacob Byler. They are homozygous for mutations in the ATP8B1 gene that encodes FIC1, an ATP-dependent enzyme with putative aminophospholipid translocase activity. Biliary canalicular membranes are asymmetrical with respect to their component phospholipids. Phosphatidyl choline predominates on the canalicular surface whereas phosphatidyl ethanolamine and sphingomyelin, both aminophospholipids, are enriched on the cellular surface. Since only phosphatidyl choline is a major component of bile, the active transport of other phospholipids from the canalicular surface seems plausible. Alternatively, FIC1 may function in bile acid transport, with different substrate specificity to BSEP.

Mutations in FIC1 cause cholestasis that is intermittent initially, later persistent with consequent cirrhosis. Bile-salt mediated diarrhea is also observed, due to lack of function in the intestine. GGT, usually a sensitive marker of cholestasis, is paradoxically low despite the presence of profound impairment of bile flow in FIC1 disease. Activity of the FIC1 P-type ATPase may be necessary for the correct localization of the bile salt export pump BSEP. Without bile salts in the biliary canaliculi, GGT remains anchored to the biliary canalicular membrane via the lipid glycosylphosphatidylinositol. Because the membrane anchor is lipid, GGT is susceptible to the detergent actions of bile salts.

BRIC or benign recurrent intra-hepatic cholestasis is also caused by mutations in FIC1. It was described in 1959, 10 years before the initial report of Byler disease. BRIC attacks are self-limited and do not lead to cirrhosis. The locus of the gene mutated in BRIC was mapped in 1995. Later, Byler disease was mapped to the same area. Before the precise identification of the responsible gene, PFIC type 1 and BRIC were considered completely separate disorders. We now know that they share a pathogenetic mechanism and that clinical variability in expression and penetrance is explained by genetic variability. Thus, the phenotypic differences are explained by different mutations having a less severe expression than in Byler disease.

BSEP bile salt export pump:
Progressive familial intra-hepatic cholestasis type 2 is caused by mutations in BSEP, the bile salt export pump encoded by the ABCB11 gene. The disease manifestations are more severe than in PFCI type 1. Jaundice is persistent with rapid progression to cirrhosis. Prior to demonstration of mutations in BSEP, PFI type 2 was distinguished from type 1 by its clinical manifestations. The liver histology was that of a giant cell hepatitis rather than ductular proliferation and the bile was more filamentous and amorphous in appearance compared with the granular bile of type 1 disease. In addition, there was no diarrhea. BSEP is only expressed in the liver and is limited to the canalicular membrane. FIC1 is expressed predominantly in the liver and intestine but also in non-gastrointestinal tissues at lower levels. Intestinal expression is related to the diarrhea which does not improve with liver transplantation and can in fact become more severe with reconstitution of normal bile flow.

HSD3B7 3β-hydroxy delta 5 C27 steroid oxidoreductase:
Progressive familial intra-hepatic cholestasis type 4 is caused by mutations in HSD3B7, a key enzyme in the bile acid synthetic pathway. Since no bile acids are secreted and bile acid precursors accumulate intra-cellularly, the manifestations are similar to those in PFIC types 1 and 2, with low GGT and progressive cholestatic liver disease. Technically, it is a disease of bile acid synthesis rather than excretion.

Hepatic excretion of phospholipid:
PFIC type 3 was initially recognized by clinical differences from types 1 and 2. The most striking was the high GGT level. In addition, the liver histology was that of ductular proliferation and inflammation despite patent bile

ducts. Furthermore, the patients are usually older at presentation and the disease process is slower. Recognition of similarities in the liver disease phenotype of a mouse model was critical in identifying the responsible gene, protein and pathogenetic mechanisms. In addition to PFIC type 3, MDR3 mutations are associated with intra-hepatic cholestasis of pregnancy and gallstones.

MDR3 multidrug resistance type 3:
Progressive familial intra-hepatic cholestasis type 3 is caused by mutations in MDR3, an ATP-binding cassette transporter encoded by ABCB4. In mice with targeted disruption of the murine equivalent, there is progressive liver disease in a biliary pattern. MDR3 is a phospholipid translocator, responsible for the biliary excretion of phosphatidyl choline. Serum lipoprotein patters in MDR3 deficiency are different than those observed in the other PFIC types. Specifically, no lipoprotein X is present. Formation of lipoprotein X is dependent on the function of MDR3.

Biliary phospholipids are decreased in PFIC type 3 patients. The ratio of bile salts to phospholipid and cholesterol to phospholipid are abnormal. Lack of phospholipids results in bile that is more detergent with the potential to damage cholangiocytes. An increased ratio of cholesterol to phospholipid is associated with cholesterol gallstone formation. Mutations in MDR3 with subtle effects on protein function may increase the incidence of gallstone formation.

In heterozygote mothers of children with PFIC type 3, intra-hepatic cholestasis of pregnancy can occur. How commonly this explains the development of symptoms during pregnancy is not yet known.

SUMMARY: Genetics of Bile Formation and Secretion

Component	Gene(s)	Product	Genetic Disorder(s)
Unknown	ATP8B1 **602397*	? ATP-dependent aminophospholipid translocase	Progressive familial intra-hepatic cholestasis type 1 = Byler disease *#211600* Benign recurrent intra-hepatic cholestasis (BRIC) *#243300*
Bile salts	ABCB11 **603201*	Bile salt export pump	Progressive familial intra-hepatic cholestasis type 2 *#601847*
Cholesterol	ABCG5 **605459* & ABCG8 **605460*	Sterol transporter	Sitosterolemia *#210250*
Phospholipid	ABCB4 **171060*	Multidrug resistance type 3	Progressive familial intra-hepatic cholestasis type 3 *#602347* Intrahepatic cholestasis of pregnancy *#147480*
Unknown	JAG1 *+601920*	Jagged1 (in Notch signaling pathway)	Alagille syndrome *#118450*

The numbers in italics refer to OMIM Online Mendelian Inheritance in Man entries
* = gene of known sequence
# = descriptive entry, usually of a phenotype, does not represent a unique locus
% = a confirmed mendelian phenotype or phenotypic locus for which the underlying molecular basis is not known
+ = description of a gene of known sequence and a phenotype

Other genetic causes of jaundice and cholestasis:
The Online Mendelian Inheritance in Man contains 148 entries mentioning either jaundice or cholestasis. In some cases, it is only a cross-reference to a related gene or protein. However, in many cases there is a defined gene and protein that causes liver disease.

Other Genetic Disorders with Jaundice or Cholestasis				
Gene	**OMIM**	**Protein**	**Genetic Disorder**	**OMIM**
PI	+107400	α1-antitrypsin	AAT, α1-antitrypsin deficiency	+107400
JAG1	+601920	Jagged 1 (Notch ligand)	AGS, Allagille syndrome	#118450
VPS33B	*608552	Vacuolar protein sorting 33, yeast, homolog of, B	ARC, Arthrogryposis, renal dysfunction and cholestasis	#208085
CFTR, ABCC7	*602421	Cystic fibrosis transmembrane conductance regulator	CF, Cystic fibrosis	#219700
HFE	+235300	HFE	HFE, Hemochromatosis	+235300
ATP7B	*606882	ATPase, Cu^{2+} transporting	WD, Wilson disease	#277900
HAMP	*606464	Hepcidin anti-microbial peptide	JH, Juvenile hemochromatosis HFE2B, Hemochromatosis type 2B	#602390
TFR2	*604720	Transferrin receptor 2	HFE3, Hemochromatosis type 3	#604250
HJV	*608374	Hemojuvelin	JH, Juvenile hemochromatosis HFE2A, Hemochromatosis type 2A	#602390

The numbers in italics refer to OMIM Online Mendelian Inheritance in Man entries
* = gene of known sequence
# = descriptive entry, usually of a phenotype, does not represent a unique locus
% = a confirmed mendelian phenotype or phenotypic locus for which the underlying molecular basis is not known
+ = description of a gene of known sequence and a phenotype

CLINICAL PRESENTATIONS: HYPERBILIRUBINEMIA, JAUNDICE AND CHOLESTASIS

The symptoms and signs are determined by the etiology - hemolysis, genetic defects, liver or biliary disease.

Jaundice is perceived when the serum bilirubin level exceeds 2-3 mg/dl. If the level of conjugated bilirubin rises, it is excreted in the urine when kidney function is normal and results in a darkening of the normal urine color. Dark urine is not pathognomonic for bilirubin. Hemoglobin, myoglobin and metabolic products excreted in patients with tyrosinosis, alkaptonuria, melanoma and porphyria can cause dark urine.

The color of normal urine is due to its content of urochrome, a compound of urobilin with a peptide of unknown structure. Urobilin, a uroporphyrin, is derived from bilirubin by bacterial deconjugation and degradation of bilirubin diglucuronide in the intestine. Urobilinogen, the initial product, is colorless. Urobilin, formed by oxidation of urobilinogen, is yellow. Urobilin is excreted in the stool or reabsorbed and excreted in bile or in the urine. Measurement of urinary urobilinogen is not a reliable indicator of bilirubin metabolism.

When no bile reaches the intestine, as in complete obstruction of the biliary tract, stool color becomes light. The color of normal stool color is due to its content of urobilin or stercobilin both of which are dived from bilirubin diglucuronide. Stercobilin is a colored oxidation product from stercobilinogen. Stercobilinogen is a reduction product of urobilinogen. Neither the presence nor the absence of pale stools is useful in determining the answer to the question "Why am I yellow?"

Cholestasis often presents with pruritus. When bile flow is obstructed, there is retention of "pruritogenic" factor and subsequent itching. Pruritus is not even nearly pathognomonic for cholestasis, since many local skin conditions including drug reactions, insect bites, uremia and various other dermatologic processes are associated with pruritus. The exact cause of pruritus in cholestasis is unknown and may represent the individual response to a variety of factors. Infusion of bile acids does not reproduce the pruritus (considered potentially causal because bile acid-binding resins can improve symptoms); morphine-like opioids may be responsible since treatment with morphine antagonists can be useful in some cases.

Because bile acids function in the absorption of fat and fat-soluble vitamins, cholestasis that is either severe or prolonged may be complicated by fat malabsorption, resulting in steatorrhea (foul-smelling, floating, bulky stools containing unabsorbed dietary fat). Deficiencies of fat-soluble vitamins can lead to night blindness (vitamin A or retinol deficiency), prolonged bleeding (vitamin K-dependent synthesis of coagulation factors), bone pain and pathologic fracture (vitamin D deficiency) and neurologic deficits (vitamin E deficiency, particularly in pediatric population). The signs of cholestasis include excoriations (scratching), increased plasma cholesterol levels and accumulation of abnormal lipoproteins (lipoprotein X) with formation of xanthelasma and xanthomas, icteric skin and eyes, and the findings of fat-soluble vitamin deficiency (night blindness, neurologic deficits, osteomalacia, prolonged bleeding). They are relatively uncommon, except for pruritic excoriations unless the cholestasis is severe and/or prolonged.

INVESTIGATIONS

Hemolysis and decreased hepatic uptake lead to an isolated increase in unconjugated bilirubin. Investigations concentrate on defining the bilirubin components present and the underlying cause. Unconjugated bilirubin is also referred to as free bilirubin or indirect-reacting bilirubin because of the necessity to add alcohol for a positive van den Bergh reaction. In conjugation defects, there is an increase in unconjugated bilirubin; a measurable decrease in conjugated bilirubin may not be detected if the assay lacks sensitivity and/or specificity.

van den Bergh Reaction

Bilirubin + Diazotized Sulfanilic Acid = No Color Change
+ Alcohol = Color Change

***Indirect-reacting** (needs alcohol)*

Bilirubin Glucuronide + Diazotized Sulfanilic Acid = Color Change

***Direct-reacting** (doesn't need alcohol)*

When the increase is only in unconjugated (non-polar, lipid-soluble) bilirubin, circulating largely bound to albumin, there is no bile excreted in the urine (hence the term acholuric jaundice, *no bile in the urine*). The excessive red cell destruction can be demonstrated as a fall in haptoglobin to undetectable levels if the hemolytic process is intravascular. The bone marrow responds by increasing the synthesis of red cells (reticulocytes increase in the circulation) and if the hemolysis is greater than red cell production rates, the red cell number falls (anemia).

Table 1: **Serum Bilirubin Levels in Isolated Hyperbilirubinemia and Jaundice**

Condition	**Unconjugated Bilirubin**	**Conjugated Bilirubin**
Normal	≤1.3 mg/dl**	≤0.3 mg/dl***
Hemolysis	Increased	Normal
Decreased uptake	Increased	Normal
Conjugation defect	Increased	<Normal
Excretion block	Increased	Increased
Liver or biliary disease*	Increased	Increased
Liver disease* plus hemolysis	Increased	Increased
* Other abnormalities present	= Indirect-reacting bilirubin = Free bilirubin ** PMH normal	= Direct-reacting bilirubin *** Methodologic ULN

The signature of cholestasis is the finding of increased levels of serum bile acids and the appearance of the abnormal lipoprotein, lipoprotein X, in the blood. However, these measurements are not routinely available. Bilirubin levels rise in cholestatic diseases if there is complete obstruction, very extensive replacement of the

parenchymal cells (hepatocytes) of the liver by the process e.g. malignant or infectious, or if the disease is widespread and/or extensive e.g. late stage of primary biliary cirrhosis.

In cholestasis, the common pattern of hepatic enzyme abnormalities is a predominant increased alkaline phosphatase (AP or alk. phos.) with a parallel increase in γ-glutamyl transpeptidase (GGT). The aminotransferases (AST, aspartate aminotransferase and ALT, alanine aminotransferase) may be elevated, but the increase is generally modest. Autoimmune markers can be positive e.g. anti-mitochondrial antibody (AMA) in primary biliary cirrhosis, perinuclear staining of anti-neutrophil cytoplasmic antibody (pANCA) in primary sclerosing cholangitis.

Imaging studies are used to determine the level of obstruction. Screening radiologic evaluation by ultrasound is the most cost-beneficial initially. Delineation of the precise nature of the obstruction may require cholangiography; endoscopic retrograde cholangiopancreatography (ERCP), percutaneous trans-hepatic cholangiography or magnetic resonance cholangiography (MRC) can be used. When extra-hepatic causes have been excluded, a liver biopsy may be needed to differentiate between various intra-hepatic causes such as primary biliary cirrhosis, drug reactions, granulomas and infiltrative processes.

When the pattern of liver test abnormalities is less specific, i.e. neither clearly hepatitic nor unequivocally cholestatic, the list of appropriate tests broadens considerably. Chronic hepatitis serologies (hepatitis B surface antigen, anti-hepatitis C virus antibody), autoimmune serologies (anti-SMA, anti-LKM1) and tests for genetic liver disease should be considered. In hemochromatosis, iron studies (serum iron, total iron binding capacity and serum ferritin) are used for screening purposes and genetic markers (homozygous mutation in the HFE gene) are sought for specific diagnosis. Wilson's disease is diagnosed by the presence of a constellation of abnormalities in copper metabolism (low ceruloplasmin, elevated urine and liver copper). When α1-antitrypsin deficiency is associated with liver disease, there is lung disease, levels of α1-antitrypsin are low and there is an abnormal genotype (ZZ instead of MM).

DIFFERENTIAL DIAGNOSIS OF HYPERBILIRUBINEMIA, JAUNDICE AND CHOLESTASIS

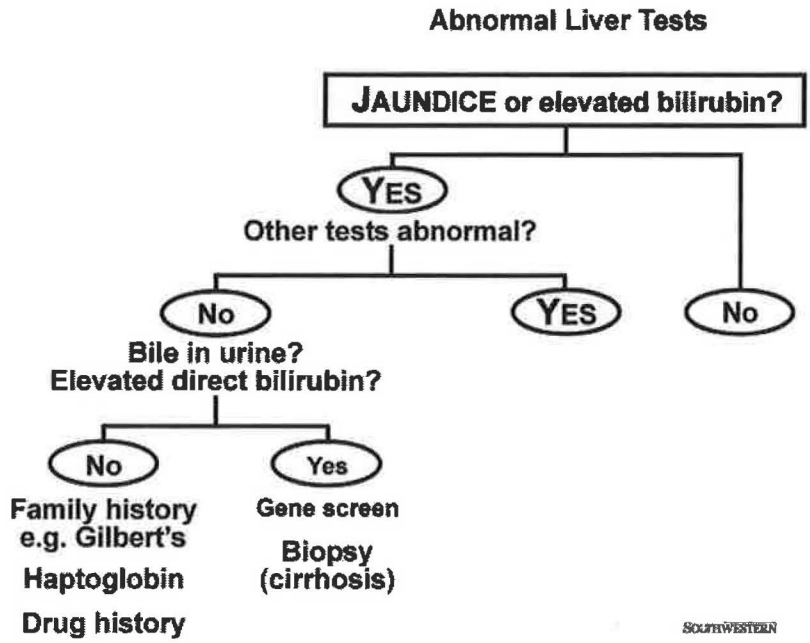
Hyperbilirubinemia can be categorized as *pre-hepatic* i.e. increased red cell destruction, *hepatic* i.e. defects in uptake, conjugation or excretion of bilirubin from hepatocytes and *post-hepatic* i.e. obstruction to bile flow anywhere along the length of the bile duct system, from the biliary canaliculus to the ampulla of Vater. When the results of laboratory tests demonstrate an isolated increase in bilirubin; there is no evidence of liver or biliary disease - neither hepatic inflammation (normal ALT and AST) nor biliary obstruction (normal alkaline phosphatase and γ-glutamyl transpeptidase). Consequently, the differential diagnosis is limited to defects in the pathway of bilirubin formation and metabolism, the *pre-hepatic* and *hepatic* categories. The differential diagnosis is also greatly affected by the age of the patient. Genetic defects are uncommon or rare in the older population but will be more likely when jaundice is present at birth.

1. **Pre-hepatic hyperbilirubinemia = hemolysis:**
Either intravascular hemolysis or extravascular hemolysis may lead to excessive red cell destruction and increased production of bilirubin from heme by the reticulo-endothelial (phagocytic) cells. When there is an increase in bilirubin production because of hemolysis of red cells, the bilirubin level does not rise until the capacity of hepatic uptake, conjugation and/or excretion is exceeded. The normal mature liver has a reserve capacity of ~7-fold. However, if this normal reserve is either exceeded or diminished by a disease process, then circulating bilirubin levels will increase. When there is no associated liver disease, merely excess hemolysis, there is only an increase in unconjugated (indirect) bilirubin. Conjugated (direct) bilirubin levels are normal.

Genetic defects in red cell enzymes such as pyruvate kinase deficiency may result in sufficient increased bilirubin load from hemolysis such that uptake, conjugation or excretion of bilirubin cannot maintain a normal serum bilirubin level.

2. **Isolated hepatic hyperbilirubinemia** (i.e. without liver or biliary disease)**:**
If there is decreased hepatic uptake of bilirubin, then unconjugated bilirubin levels rise and conjugated bilirubin levels are normal, as with hemolysis. This can occur in drug reactions, where there is competition for bilirubin

uptake e.g. rifampin, probenecid. Decreased hepatic uptake (as well as a conjugation defect) may be present in persons with Gilbert syndrome.



Figure

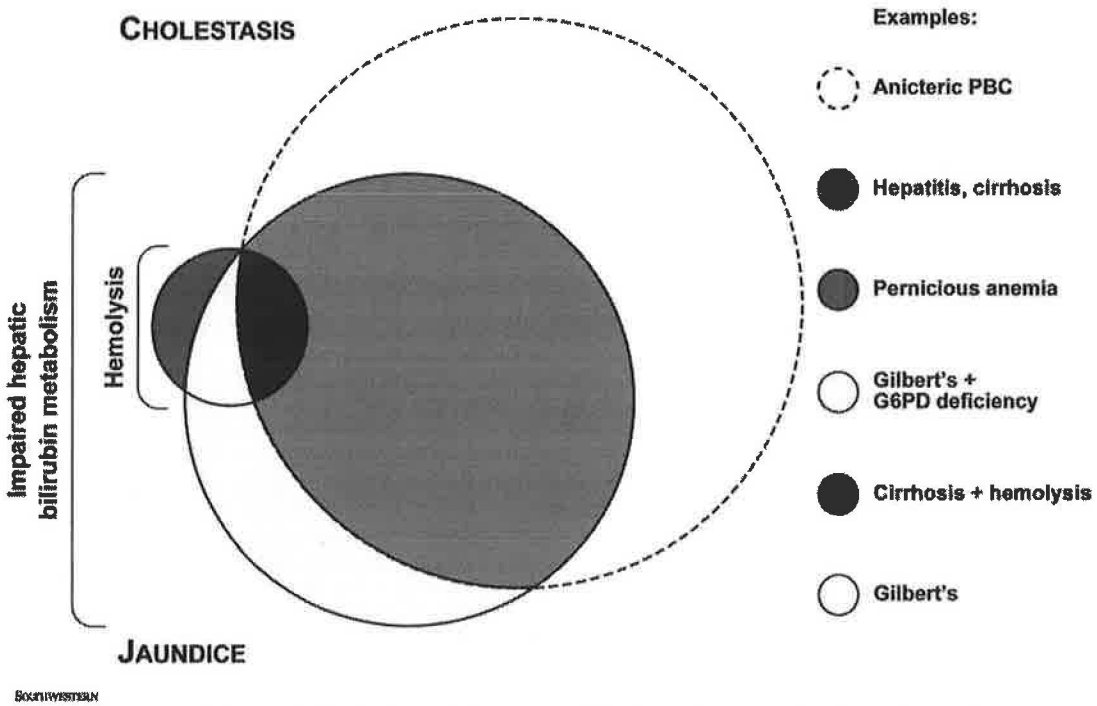
3. **Hepatic and post-hepatic hyperbilirubinemia** (with liver or biliary disease):
If the pattern of hepatic enzyme elevation is cholestatic in nature i.e. alkaline phosphatase increased relatively more than aminotransferases, the major diagnostic possibilities are causes of intra-cellular, intra-hepatic and extra-hepatic obstruction. A large number of disease processes affecting the liver manifest less distinctive patterns of abnormalities but may, if severe enough, cause jaundice. These include chronic viral hepatitis, chronic hepatitis from drugs, toxins (including alcohol), or autoimmune-mediated and genetic diseases having hepatic presentations.

DIAGNOSIS

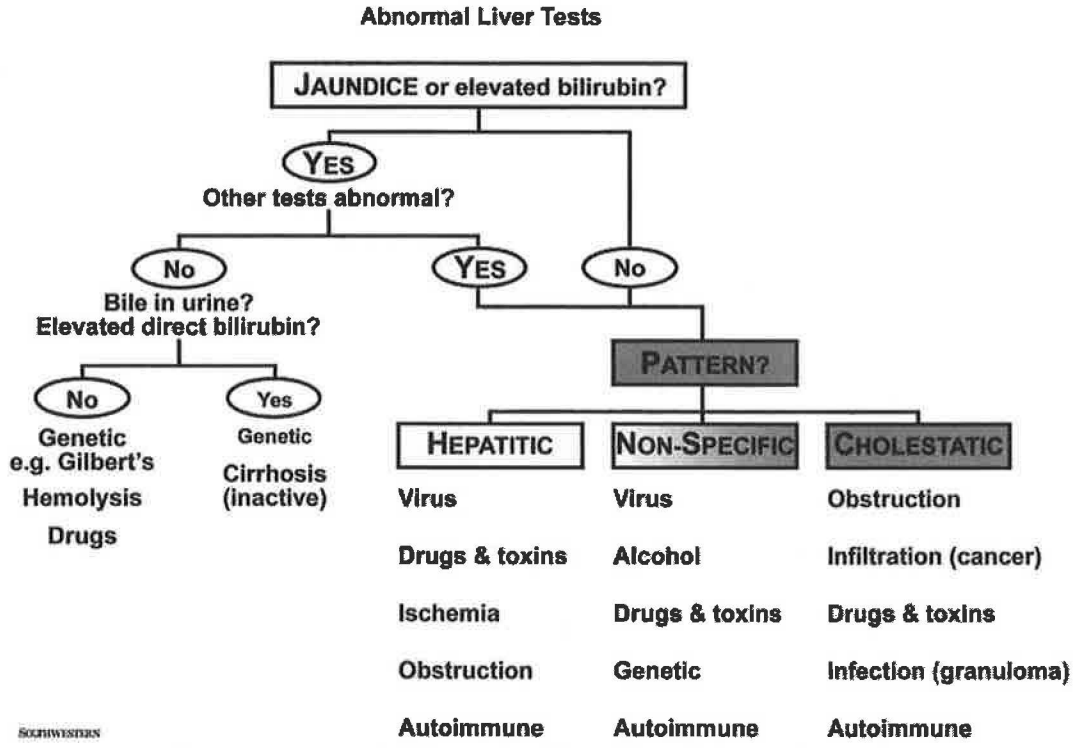
If the patient has an isolated increase in serum bilirubin, the next step is to determine whether this is a pure, unconjugated hyperbilirubinemia or mixed (unconjugated + conjugated). Two complementary tests are helpful - bilirubin fractionation and urinalysis (for presence of bilirubin, reported as bile at PMH). Bilirubin fractionation measures conjugated and unconjugated bilirubin separately. If there is isolated, unconjugated hyperbilirubinemia, a haptoglobin is helpful in assessing intra-vascular hemolysis (results in undetectable haptoglobin) and a peripheral smear may show abnormal red cells that are subject to hemolysis. If there is an increase in conjugated (water-soluble) bilirubin in the serum, then, with few exceptions, the test for bile in the urine will be positive. Remember, conjugated bilirubin is not elevated alone, both unconjugated and conjugated bilirubin will be elevated. The relative magnitude of the increase of the separate fractions is not useful in determining causation.

The etiology of cholestatic jaundice is determined by the combination of: i). a careful history for exposures to potentially hepatotoxic compounds (prescription drugs, over-the-counter and herbal remedies or nutritional supplements); ii). appropriate serologic tests (autoimmune markers); iii). imaging studies (abdominal sonography for gallstones and bile duct dilation 2° to obstruction); and iv). liver biopsy if necessary.

Summary:
Jaundice develops when the bilirubin load exceeds the capacity of hepatic uptake, conjugation and/or excretion. Common etiologies include hemolysis (increased bilirubin load), Gilbert syndrome (conjugation defect plus ? another factor), acute hepatitis (diminished uptake, conjugation and excretion), intra-cellular and intra-hepatic cholestasis (from drugs, infection, inflammation, immune-mediated cellular destruction) and extra-hepatic obstruction (stones, strictures, tumors).

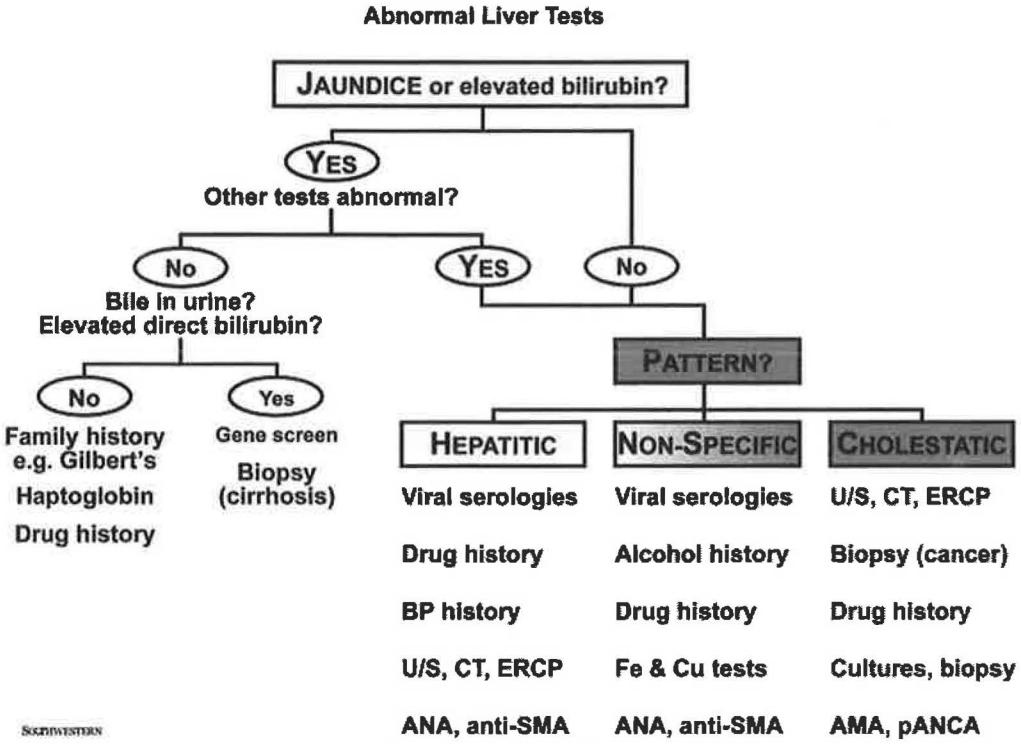


Summary Figure 8: Relationship between Cholestasis, Hemolysis and Jaundice



Summary Figure 9: Diagnosis of Jaundice – Disease Categories

SUMMARY: Diagnosis of Jaundice
"Hepatitic" liver disease (aminotransferases 500 IU/L) Acute hepatitis - acute viral serologies, anti-SMA (autoimmune) Drugs and toxins (NOT alcohol alone) - check history & Rx Ischemia and heat stroke (poor perfusion) - low BP? Acute obstruction (choledocholithiasis) - ultrasound
Cholestatic liver disease (increased alkaline phosphatase) Extra-hepatic obstruction: e.g. strictures, cancers & stones Intra-hepatic obstruction: e.g. PBC, PSC, granulomas, infiltrative processes, drugs Ultrasound, CT, MR cholangiography, ERCP Autoimmune markers (AMA in PBC, pANCA in PSC), ERCP
Non-specific liver disease (aminotransferases <500 IU/L) Viral hepatitis - chronic viral serologies Drugs and toxins (alcohol alone) - check history & Rx Autoimmune liver disease - ANA, anti-SMA Sepsis (cytokine-mediated interference in bile secretion) without hypoperfusion
Genetic liver disease (liver biopsy) Hemochromatosis - iron studies, *HFE* gene mutation α1-antitrypsin (AT) deficiency - α1AT levels Wilson('s) disease - copper studies, ceruloplasmin
AMA, anti-mitochondrial antibody; ANA, anti-nuclear antibody; ERCP, endoscopic retrograde cholangiopancreatography; HFE *genetic locus for hereditary hemochromatosis pANCA, perinuclear anti-nuclear cytoplasmic antibody; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; SMA, smooth muscle antibody*



Summary Figure 10: Diagnosis of Jaundice – Useful Tests and Strategies

GLOSSARY

ABCC2 C2 member of the ABC (ATP binding cassette) superfamily; symbol for MRP2, canalicular conjugate export pump
acholuric no bile in the urine, e.g. acholuric jaundice = unconjugated hyperbilirubinemia; from the Greek *a* negative, *chole* bile and *ouron* urine
Alagille *syndrome* multisystem genetic disorder of bile duct paucity in combination with cardiac, ocular, and skeletal abnormalities and characteristic facial appearance; autosomal dominant with highly variable expressivity; due to mutations in JAG1; gene product, Jagged1, involved in Notch signaling pathway; Daniel *Alagille*, French pediatrician, born 1925
ALT alanine aminotransferase (glutamic-pyruvic transaminase, serum GPT); more liver-specific than AST
AMA anti-mitochondrial antibody, recognizes the E2 complex of mitochondrial pyruvate dehydrogenase; associated with primary biliary cirrhosis
ANA anti-nuclear antibody; associated with autoimmune hepatitis
AP alkaline phosphatase; sources include liver, bone and placenta
AST aspartate aminotransferase (glutamic-oxaloacetic transaminase, serum GOT); sources include liver, muscle, red cells
A-SMA, ASMA anti-smooth muscle antibody, recognizes F-actin; associated with autoimmune hepatitis
BRIC *acronym* benign recurrent intra-hepatic cholestasis due to mutations in FIC1 gene (different mutations than Byler disease)
BSEP bile salt export pump, mutated in progressive familial intra-hepatic cholestasis type 2 PFIC2
BSP bromosulfophthalein used in testing hepatic function; organic anion transported into hepatocytes and excreted in bile
Byler *disease* familial intra-hepatic cholestasis type 1 due to mutations in FIC1 gene; Amish kindred descendents of Jacob *Byler*
cholestasis result of impairment to normal bile flow; may be intra-cellular, intra-hepatic or extra-hepatic in origin; from the Greek *chole* bile and *stasis* stoppage
canals of Hering communicate between hepatocyte bile canaliculi and cholangioles, the terminal bile ductules; walls are hepatocytes on one side and cholangiolar cells on the other side; Karl Ewald Konstantin *Hering* German physiologist, 1834-1918, Hering's law, test and theory, father of Heinrich Ewald Hering, Hering's nerve
http://www.whonameit.com
cMOAT *acronym* (see-moat) canalicular Multispecific Organic Anion Transporter; transports bilirubin glucuronide across the hepatocyte apical membrane into the biliary canaliculus; defective in Dubin-Johnson syndrome; ≡ MRP2
conjugated bilirubin *cmpd. n.* bilirubin mono- or di-glucuronide; bilirubin that has been conjugated with glucuronide by UDP-glucuronyl transferase in the liver; direct-reacting bilirubin
conjugation joining together; from the Latin *conjugatio* a blending
Dubin-Johnson *syndrome* conjugated hyperbilirubinemia with intra-hepatic accumulation of brown, coarsely granular pigment, due to MRP2 (cMOAT) mutation; Isidore Nathan *Dubin*, American pathologist, 1913-1981; Frank B. *Johnson*, American pathologist, born 1919
ERCP endoscopic retrograde cholangiopancreatography
excoriations scratch marks; from the Latin *excoriare* to flay, *ex* out, *corium* skin
FIC1 P-type (type IV) ATPase, putatively an aminophospholipid translocase that flips phosphatidylserine and phosphatidyl ethanolamine (transporter symbol *ATP8B1*)
GGT also GGTP, gamma glutamyl transpeptidase
Gilbert syndrome *disorder* common, mild unconjugated hyperbilirubinemia, bilirubin increases with stress, fasting etc; UDPGT promoter mutation plus another alteration in bilirubin metabolism e.g. mild hemolysis; Nicolas Augustin *Gilbert*, French physician, 1858-1927
haptoglobin circulating plasma protein that binds free hemoglobin and thence is cleared by the liver; hepatic origin, acute phase reactive; from the Greek *hapto* hold fast
HFE hereditary hemochromatosis gene, HFE = gene product
HUGO *acronym* HUman Genome Organisation http://www.gene.ucl.ac.uk/hugo/
icterus synonymous with jaundice; from the Greek *ikteros* jaundice; Orioles have bright yellow bodies, the genus is *Icterus*.

ISBT Ileal **S**odium **B**ile acid co**T**ransporter; transports bile acids from the intestinal lumen, permitting enterohepatic circulation
jaundice yellow (skin, eyes, mucous membranes); from the Latin *galbinus*, German *gelb*, French *jaune* yellow
kernicterus (unconjugated) bilirubin encephalopathy; German for "nuclear jaundice"
ligandin glutathione-S-transferases, ubiquitous chaperone-type protein in hepatocytes; from the Latin *ligare* to bind
lipoprotein X abnormal circulating lipoprotein in cholestatic syndromes; from the Greek *lipos* fat
mdr2 murine equivalent of MDR3
MDR1 and 3 Multi**D**rug **R**esistance type 1 and 3 gene products in man; P-glycoprotein family members involved in ATP-dependent transport; type 3 – transports phospholipid into bile; defective in rare familial cholestatic syndromes (progressive familial intra-hepatic cholestasis type 3, PFIC3)
MM normal alpha-1 anti-trypsin genotype
MRP2 Multidrug **R**esistance-associated **P**rotein 2 gene product in man; transporter symbol *ABCC2*; transports bilirubin conjugates, sulfates, glutathione conjugates across the hepatocyte apical membrane into the biliary canaliculus, using ATP as energy source; defective in Dubin-Johnson syndrome; ≡ cMOAT
NTCP sodium [**N**a]-**T**aurocholate **C**otransporting **P**olypeptide; transports bile acids from circulation into the hepatocyte
OATP2, OATP-C Organic **A**nion **T**ransporting **P**olypeptide 2 or C, transporter for sodium-independent uptake of organic anions; many substrates shared by other OATPs; transporter symbol *SLC21A6*, member of gene family *SLC21A* within the gene superfamily of solute carriers (SLC), Human Gene Nomenclature Committee Data Base http://www.gene.ucl.ac.uk/nomenclature/
OMIM online Mendelian Inheritance in Man, one of the resources of NCBI, the National Center for Biotechnology Information and NLM, the National Library of Medicine which include PubMed, Blast and Entrez; http://www.ncbi.nlm.nih.gov/entrez/query.fcgi
PBC primary biliary cirrhosis
PFIC *acronym* (pee-fick) progressive familial intra-hepatic cholestasis, types 1 (FIC1), 2 (BSEP) and 3 (MDR3); from mutations in *FIC1*, *BSEP* and *MDR3* genes
PSC primary sclerosing cholangitis
pruritus itching; from the Latin *prurire* itch
PT prothrombin time, a measure of clotting factors in the extrinsic pathway, including vitamin K-dependent factors II, VII and X as well as I and V
Rotor syndrome *disorder* chronic familial non-hemolytic jaundice, differing from Dubin-Johnson by lack of pigment; Arturo B. *Rotor* 20th century Philippine physician
UDPGT, UDP-glucuronosyl transferase *enzyme* uridine diphosphate-glucuronyl transferase, catalyses conjugation of bilirubin with glucuronide; defective in Crigler-Najjar syndromes
unconjugated bilirubin *cmpd. n.* bilirubin that has not been conjugated with glucuronide by UDP-glucuronyl transferase; indirect-reacting bilirubin
van den Bergh reaction *cmpd. n.* distinguishes unconjugated from conjugated bilirubin; A. A. Hymans *van den Bergh* Dutch physician, 1869-1943
xanthelasma xanthomas affecting eyelids, soft yellow spots or plaques; from the Greek *xanthos* yellow and *elasma* plate
xanthoma papule, nodule or plaque of yellow color; from the Greek *xanthos* yellow
ZZ alpha-1 anti-trypsin genotype, phenotypically associated with lung and liver disease

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