Genetic Testing in Liver Disease

What to Order, in Whom, and When

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KEYWORDS

- Genetic testing • Hemochromatosis • Wilson disease
- Progressive familial intrahepatic cholestasis
- Benign recurrent intrahepatic cholestasis • Lysosomal acid lipase deficiency
- Gilbert syndrome • Alpha-1 antitrypsin deficiency

KEY POINTS

- The most common cause of hereditary hemochromatosis is a C282Y mutation in the HFE gene with a penetrance of 10% to 52%.
- Gilbert syndrome is a common and benign cause of indirect hyperbilirubinemia with no signs of hemolysis and no associated liver injury.
- Progressive familial intrahepatic cholestasis is a rare cause of chronic cholestasis in children and young adults. Benign recurrent intrahepatic cholestasis is a benign cause of recurrent cholestasis seen in both adults and children.
- Alpha-1 antitrypsin deficiency causes both lung and liver disease. It is the most common genetic cause of liver disease in children.
- Wilson disease can cause neurologic disease and liver disease. Patients between the ages of 3 and 55 years with any acute or unexplained chronic liver disease should be tested for Wilson disease.

INTRODUCTION

When evaluating a patient with abnormal liver function tests, investigating genetic causes of liver disease is an important part of the work-up. The initial evaluation of a patient with abnormal liver function tests includes a history and physical examination. Family history plays a critical role because it can help determine which patients to consider for genetic testing. The age of onset of abnormal liver function tests and the pattern of abnormal liver function tests, hepatocellular or cholestatic, play a role

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in what testing is performed. This article evaluates common genetic causes of liver disease, when and in whom to test for them, and what tests to order.

HEREDITARY HEMOCHROMATOSIS

Hemochromatosis occurs from the unregulated transfer of iron from the intestine into the blood leading to toxic levels depositing in various organs and is usually caused by an underlying genetic disorder.[1] Most (80%–90%) cases of hereditary hemochromatosis are caused by an autosomal recessive mutation in the *HFE* gene, C282Y.[2–8] Two less common mutations are H63D and S65C, which usually only cause signs and symptoms of iron overload when present as compound heterozygotes with C282Y.[2,4,9] In patients with liver disease, 3% to 5.3% are homozygous for C282Y and therefore testing for hemochromatosis should be performed in the work-up of liver disease of unknown cause or in patients with iron overload on laboratory testing or imaging.[5,9] It is especially important to test those patients who have a first-degree relative with hereditary hemochromatosis as well as those patients with imaging studies that show iron overload.[2,5,8] At diagnosis, about 4.4% to 11.8% of C282Y homozygous male patients have cirrhosis and 0% to 2.7% of C282Y female patients have cirrhosis.[1,7,10] Therefore early diagnosis is important because initiation of phlebotomy before the development of cirrhosis can reduce or stop the progression of hereditary hemochromatosis.[2,10]

Hereditary hemochromatosis is most commonly seen in Caucasian of Northern European descent.[2,6,9] About 6% to 10% of Caucasian have 1 allele for C282Y and 1 in 250 to 300 Caucasian have 2 alleles.[1,4,5] Although the HFE gene mutations are fairly common, only 10% to 52% of patients homozygous for C282Y develop clinical signs of iron overload.[1,2,5–8,11] Manifestations of hereditary hemochromatosis are more prevalent in men and present earlier in men, likely in part because of menstruation and therefore iron loss in women.[2,5,6,8,10] Patients with hereditary hemochromatosis can show other symptoms of iron overload that may prompt testing, such as chondrocalcinosis, diabetes mellitus type 1, heart failure, or porphyria cutanea tarda[1,2,5,6] Up to 19% of patients with porphyria cutanea tarda are homozygous for C282Y.[5,8,12]

The initial screening tests for hereditary hemochromatosis include blood tests for ferritin and transferrin saturation, which is calculated from iron/total iron binding capacity.[2,5] Transferrin saturation greater than 45% should prompt genetic testing for the most common causes of hereditary hemochromatosis.[1,2,4–6,9] An increased ferritin level is also expected in hereditary hemochromatosis, but is not highly specific, thus an increased ferritin level with a normal transferrin saturation is not common in hereditary hemochromatosis and should lead to investigation into alternative causes of liver disease.[1,13] In hereditary hemochromatosis, a ferritin level is still useful because of its high sensitivity and because a level greater than 1000 μg/L can help predict patients who have advanced fibrosis.[2,10] Testing for advanced fibrosis or cirrhosis should be performed in patients who are homozygous for C282Y or compound heterozygotes for C282Y who have a ferritin greater than 1000 μg/L, hepatomegaly, age more than 40 years, or abnormal liver tests.[2,5,6,10,14] A liver biopsy is not always necessary because MRI can evaluate for cirrhotic morphology and can quantify the amount of iron in the liver, and transient elastography can also be used to evaluate for advanced fibrosis.[5,15,16]

In patients with laboratory testing consistent with iron overload, but who are not C282Y homozygotes, other causes of liver disease should be considered.[1,2,15] Iron

overload is common in other liver diseases, such as alcohol-related liver disease, nonalcoholic fatty liver disease (NAFLD) and viral hepatidites.[1,2,13,15,17] In this situation, a liver biopsy may be necessary to investigate for alternative causes of liver disease.[2] In these patients, heterozygosity or compound heterozygosity for C282Y or H63D may lead to more advanced liver disease because of increased iron stores.[3] Liver biopsies should be stained with Perls Prussian blue to determine the amount of iron and the location of iron present.[2,15] Iron overload can be seen in cirrhosis of any cause; however, in hemochromatosis, the distinguishing feature is that iron is deposited in the fibrous septa, bile ducts, and walls of the vasculature.[15] The hepatic iron concentration in a liver biopsy of at least 236 μmol/g dry weight has a sensitivity of 80% and a specificity of 78% for distinguishing patients with cirrhosis.[2,10]

If true hepatic iron overload is present, non-HFE genetic hemochromatosis should be suspected. Less common genetic causes of hereditary hemochromatosis include juvenile hemochromatosis, mutations in transferrin receptor-2, mutations in ferroportin, aceruloplasminemia, atransferrinemia, and African iron overload.[2,18] Given the rarity of these mutations, genetic testing for the disorders is infrequently performed.[1,5,15] Genetic testing is only done if laboratory analysis suggests iron overload, but *HFE* mutations and other causes of liver disease are ruled out or treated and iron overload in the liver is confirmed.[1,5,15] In contrast with *HFE*-associated hereditary hemochromatosis, ferroportin disease and aceruloplasminemia show a transferrin saturation less than 45% with an increased ferritin level and signs of iron overload.[5] These findings need to be distinguished from secondary iron overload caused by excessive dietary intake or hemolysis/ineffective erythropoiesis.

Disease	Common Laboratory Abnormalities	Presentation of Disease
Hereditary hemochromatosis (*HFE* form)	↑AST/ALT Transferrin saturation >45% ↑Ferritin Genotype analysis most commonly C282Y homozygote	Abnormal LFTs Cirrhosis Type 1 diabetes mellitus Heart failure
What test to order? *HFE* genotype		

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; LFTs, liver function tests.

GILBERT SYNDROME

Gilbert syndrome is a benign cause of unconjugated hyperbilirubinemia in people with no liver disease or hemolysis.[19] It is caused by reduced bilirubin glucuronidation, a process needed to excrete bilirubin. The prevalence is about 1.6% to 10% of the population.[19–21] The bilirubin levels can fluctuate, but are usually between 1 and 5 mg/dL, always with a normal direct (conjugated) bilirubin level.[19,20,22,23] In adults, it does not lead to clinically significant liver disease.[19,21] Patients have normal aminotransferase and alkaline phosphatase levels, and their hemolysis work-up is negative with no other physical examination findings to suggest liver disease.[19,22] In studies that have evaluated liver biopsies in these patients, normal histology was noted, and a liver biopsy is not needed for diagnosis.[22] Thus patients with isolated unconjugated hyperbilirubinemia and no other evidence of liver disease should be reassured and require no further work-up. Gilbert syndrome is more prevalent in men than in women and fasting can increase the serum bilirubin level.[19,21]

Disease	Common Laboratory Abnormalities	Presentation of Disease
Gilbert syndrome	+Unconjugated bilirubin	Fasting increases bilirubin level

PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS

Progressive familial intrahepatic cholestasis (PFIC) encompasses 3 autosomal recessive mutations that lead to chronic cholestasis: PFIC1, PFIC2, and PFIC3.[24] The incidence of PFIC is about 1 per 50,000 to 1 per 100,000 births, and it is the cause of liver disease in about 10% to 15% of children with cholestatic liver tests.[24–28] Patients with PFIC can present with jaundice, pruritus, splenomegaly, and hepatomegaly.[24,25,29] Specifically, patients with PFIC1 and PFIC2 usually present at a few months of age, whereas patients with PFIC3 usually present later in childhood or early adulthood.[24,25,29] Despite cholestatic abnormalities on their liver tests, patients with PFIC1 and PFIC2 have normal gamma-glutamyltransferase (GGT) levels, whereas patients with PFIC3 have increased GGT levels.[24,25,29] All 3 classifications of PFIC have increased serum bile acid levels.[24,25,29]

PFIC1 and PFIC3 usually present with a mild increase in alanine transaminase (ALT) level and a normal alpha fetoprotein (AFP) level, whereas patients with PFIC2 show a marked increase in ALT level to more than 5 times normal with an increased AFP level.[24,30] PFIC1 and PFIC2 often present at a few months of age with recurrent or chronic jaundice and PFIC2 usually progresses more quickly than PFIC1 to end-stage liver disease.[24,30] Hepatocellular carcinoma or cholangiocarcinoma at a very young age (<1 year old) can be seen in PFIC2 and therefore, after diagnosis, screening for malignancies with imaging modalities is important.[24–26,30] Patients with PFIC1 can have other signs of the disease, such as short height, deafness, diarrhea, pancreatitis, an increased sweat electrolyte concentration, and hepatic steatosis.[24,30] In PFIC1, a mutation in the gene ATP8B1, which encodes for the protein FIC1, not only affects the protein expression in the liver but also in the pancreas and small intestine, likely leading to the other symptoms associated with the disease.[29,31] PFIC3 usually presents with cirrhosis later in childhood or young adulthood.[24]

PFIC should be considered in children with cholestasis after more common liver diseases and biliary diseases have been excluded, such as biliary atresia, Alagille syndrome, alpha-1 antitrypsin deficiency, cystic fibrosis, sclerosing cholangitis, and biliary obstruction.[24,25,29] Serum bile acid level should be tested because increased serum bile acid levels rule out problems with bile acid synthesis.[24–26,30] Liver function tests, GGT, and imaging studies help to rule out other more common causes of liver disease before diagnosing PFIC.[24,25,27]

A liver biopsy may aid in the diagnosis of PFIC. A liver biopsy in PFIC1 and PFIC2 commonly shows canalicular cholestasis.[24,25] A liver biopsy in PFIC2 usually shows more fibrosis than is seen in PFIC1 and a giant cell hepatitis.[24,25] A liver biopsy in PFIC3 usually shows proliferation of the bile ducts and fibrosis.[24] Specialized stains can further help differentiate PFIC2 and PFIC3. The gene ABCB11 encodes the bile salt export pump (BSEP) protein, most commonly thought to be defective in PFIC2.[29] The gene ABCB4 encodes the multidrug resistance 3 (MDR3) protein, most commonly thought to be defective in PFIC3.[29] A liver biopsy can be stained for BSEP and MDR3 and therefore differentiate which type of PFIC is present.[24,25,29,30] The protein staining is expected to be low or absent in most PFIC cases; however, normal protein staining may be present if patients have a loss of function mutation with normal synthesis of the proteins, and loss of staining can

be infrequently seen in other causes of neonatal cholestasis.[24,25,29,30] Additional testing that may aid in the diagnosis of PFIC is evaluation of the composition of bile. This evaluation is usually done if cholangiography is being performed to rule out other causes of disease. A low bile salt concentration is seen in PFIC1 and PFIC2 (lower in PFIC2) and a low phospholipid level is seen in the bile in PFIC3.[24,30] The proteins mutated in PFIC1 and PFIC2 play a role in the secretion of bile, whereas the protein mutated in PFIC3 plays a role in the translocation of phospholipids into bile.[24,30]

Genetic testing can confirm the diagnosis of PFIC, although in a small number of patients the genetic defect may not be elucidated because some genes likely remain unidentified.[24,26,32] Family history is important in the evaluation of PFIC because cases of heterozygous mutations for the PFIC proteins have been found in women who developed intrahepatic cholestasis of pregnancy.[24]

Disease	Common Laboratory Abnormalities	Presentation of Disease
PFIC	+Alkaline phosphatase	Jaundice
	PFIC1: normal GGT	Pruritus
	Low bile salt concentration in bile	Splenomegaly
	PFIC2: normal GGT	Hepatomegaly
	Low bile salt concentration in bile	PFIC1: short height, deafness, diarrhea, pancreatitis, increased sweat electrolyte concentration, hepatic steatosis
	+ALT	
	+AFP	
	PFIC3: +GGT	PFIC2: HCC or cholangiocarcinoma
	Low phospholipid concentration in bile	PFIC3: cirrhosis in late childhood and young adulthood
What test to order? PFIC genotype (ATP8B1, ABCB11, ABCB4 mutations)		

Abbreviations: HCC, hepatocellular carcinoma.

BENIGN RECURRENT INTRAHEPATIC CHOLESTASIS

Benign recurrent intrahepatic cholestasis (BRIC) is an autosomal recessive disease caused by a mutation in the same genes as in PFIC; however, BRIC is a benign disease.[24] BRIC is a rare disease that can present in both childhood and adulthood and leads to recurrent episodes of cholestasis.[33] Each episode of cholestasis can last for a variable amount of time and the time between episodes can range from weeks to years.[31,34] Between episodes, the patients are asymptomatic with normal liver tests.[31,34] During the recurrent episodes of cholestasis, patients with BRIC develop jaundice, pruritus, increased serum bile acid levels, increased alkaline phosphatase levels, a conjugated hyperbilirubinemia, and a low GGT level.[31,33] BRIC, unlike PFIC, does not progress to chronic cholestasis or cirrhosis.[33]

Some patients experience symptoms before the onset of an episode of cholestasis, such as fatigue, nausea, decreased appetite, and pruritus.[31] If a liver biopsy is performed during an episode of cholestasis, it shows cholestasis but no signs of chronic liver disease.[24,31,33] In order to diagnose BRIC, the patient must have at least 2 episodes of cholestasis with a symptom-free interval and alternative causes of cholestasis must be excluded, which usually includes imaging, laboratory tests, and likely a liver biopsy.[31] Case series have shown that some patients note respiratory tract infections before the episodes of cholestasis.[31,34] The diagnosis can be supported by genetic testing, which would show a mutation in the same genes as are mutated in PFIC.[24,31]

Disease	Common Laboratory Abnormalities	Presentation of Disease
BRIC	During episodes of cholestasis: +alkaline phosphatase +Conjugated hyperbilirubinemia +Serum bile acids Normal GGT Between episodes of cholestasis: Liver tests normal	During episode of cholestasis: jaundice Pruritus

LYSOSOMAL ACID LIPASE DEFICIENCY

Lysosomal acid lipase deficiency (LAL-D) is a rare autosomal recessive disorder that leads to problems with cholesterol metabolism.[35] It is a lysosomal storage disorder caused by a lack of, or deficiency in, liposomal acid lipase,[30,31] which ultimately leads to accumulation of cholesterol in different organs and macrophages.[35,36]

LAL-D has different variations. The most severe form of LAL-D is called Wolman disease, which presents in early childhood, usually at 2 to 4 months of age, with symptoms of malabsorption, calcification of the adrenal glands, and hepatomegaly.[35–38] The symptoms of Wolman disease are caused by the location of lipid accumulation, such as in the liver, spleen, adrenal glands, bone marrow, lymph nodes, macrophages, and small intestinal villi.[35,36] Wolman disease has a high mortality in the first year of life.[36,38] Cholesteryl ester storage disease (CESD) is a different form of LAL-D that can present later in childhood or in adulthood and cause hepatosplenomegaly, dyslipidemia, accelerated atherosclerosis, and liver disease.[35,36] Adrenal calcifications can sometimes be seen in this form of the disease.[36] The different LAL-D phenotypes are based on the level of activity of lysosomal acid lipase.[36] Patients with Wolman disease have either no functioning enzyme or less than 1% activity, which is in contrast with CESD, which has a higher level of activity and therefore later onset.[36]

CESD is most common in Caucasian of European descent.[35,36] About half of all cases of CESD are caused by a mutation in the *E8SJM* gene.[20] Therefore, screening for this genotype showed an estimated prevalence of the disease to be 25 per million in the German population.[20] In North America, the prevalence of CESD is about 0.8 per 100,000 in white and Hispanic populations.[39]

Patients with LAL-D may present with abnormal liver function tests, splenomegaly, and hepatomegaly.[35,36] When evaluating cholesterol levels, these patients have high triglyceride and total cholesterol levels with low high-density lipoprotein (HDL) levels.[35,36,40] Imaging studies can help rule out other causes of liver disease and evaluate for hepatomegaly; splenomegaly; and, in Wolman disease, adrenal gland calcifications.[35,36] After being ruled out for other more common causes of liver disease, a liver biopsy in these patients can show microvesicular steatosis and birefringent cholesterol ester crystals or their remnant crystals in hepatocytes as well as lipids in the Kupffer cells.[4,35,36,38,41,42] Immunostaining can be performed on the liver biopsy specimen for lysosomal proteins and this confirms that the location of lipid deposition is the lysosomes and aids in the diagnosis.[36] The liver biopsy can be mistaken for NAFLD or cryptogenic cirrhosis, which can make it harder to diagnose adults with the disease.[35,36] A dried blood spot is used to determine the peripheral leukocyte LAL activity because a low activity would support the diagnosis of LAL-D.[4,35,36,38,41,42] Genotype analysis is not necessary for diagnosis, but a mutation in *LIPA*, the gene for LAL, is diagnostic.[35,36,40]

Disease	Common Laboratory Abnormalities	Presentation of Disease
LAL-D	+AST/ALT	Hepatomegaly
	+Alkaline phosphatase	Splenomegaly
	Decreased peripheral leukocyte LAL activity in blood	Wolman disease: Calcification of the adrenal glands
	CESD:	Symptoms of malabsorption
	+Triglycerides	
	+Total cholesterol	
	Low HDL level	
What test to order? *LIPA* genotype		

ALPHA-1 ANTITRYPSIN DEFICIENCY

Alpha-1 antitrypsin is a protease inhibitor (Pi) produced in the liver that works to inhibit neutrophil elastase, which can degrade proteins, especially in the lungs.[43–46] Alpha-1 antitrypsin deficiency is an autosomal recessive mutation that can lead to early-onset panacinar emphysema in smokers (aged 40–60 years) as well as liver disease.[43,45,46] Other rarer manifestations of alpha-1 antitrypsin deficiency include panniculitis, often at sites of trauma, and vasculitis, which is usually c-ANCA (cytoplasmic antineutrophil cytoplasmic antibody) positive.[46–48]

There are multiple variants of the alpha-1 antitrypsin genotype and some of these variants produce normal levels of alpha-1 antitrypsin, whereas some lead to reduced levels.[45] The reduced levels are associated with disease.[45] The null genotype leads to no production of alpha-1 antitrypsin.[45] The normal alpha-1 antitrypsin genotype is labeled MM and severe deficiency is ZZ.[43,49,50] The ZZ genotype most commonly leads to liver disease and accounts for about 0.1% of patients with abnormal alpha-1 antitrypsin genotypes.[43,49,50] Less commonly, the SZ genotype and the M_{malton} genotype can lead to liver disease.[45,51] The prevalence of alpha-1 antitrypsin deficiency is about 1 per 5000 to 1 per 1640 in Caucasian, and heterozygosis for the MZ phenotype occurs in about 2% to 3% of the white population.[43,44,46,49,52]

The genetic defect associated with alpha-1 antitrypsin deficiency changes the enzyme shape so that it is retained in hepatocytes and can be seen on a liver biopsy when stained with periodic acid–Schiff (PAS) stain.[43,44,53] The enzyme appears as PAS-positive, diastase-resistant globules in hepatocytes.[43,44,53]

Patients with alpha-1 antitrypsin deficiency can present with jaundice, hepatitis, hepatomegaly, and/or cirrhosis in children and with abnormal liver tests or cirrhosis in adults.[43–45,52,54] Patients can have increased bilirubin levels and serum aminotransferase levels.[45,52] In children, the liver function tests may normalize at a few months of age, but they should be followed for progression to cirrhosis.[45,52] Adults diagnosed with alpha-1 antitrypsin deficiency often do not show abnormal liver tests or liver disease in childhood.[45] Alpha-1 antitrypsin deficiency is a more common cause of liver disease in men than in women.[51,52,54]

Although alpha-1 antitrypsin deficiency is the most common genetic cause of liver disease in children, most people with this deficiency do not develop liver disease.[43,44,46,51,52] Only about 7% to 20% of children with alpha-1 antitrypsin deficiency PiZZ genotype develop liver disease.[43,44,46,52] Patients with abnormal liver function tests should be evaluated for alpha-1 antitrypsin deficiency, especially if they have a family history of liver disease or emphysema in a nonsmoker. The screening examination evaluates the level of alpha-1 antitrypsin in the blood.[43,46,49] When low levels are found or pretest suspicion is high, further testing for abnormal phenotypes or genotypes is performed.[43,46,49] The PiS and PiZ genotypes can be diagnosed via

isoelectric focusing but, if patients do not have these genotypes and still have a low alpha-1 antitrypsin level, evaluating the alpha-1 antitrypsin gene (SERPINA1) for mutations is necessary.[43,46,49] For example, the null genotype cannot be discovered by isoelectric focusing because no protein is made.[43,46,49] Alpha-1 antitrypsin is an acute phase reactant and levels can be increased in the presence of high estrogen levels, which can cause a false-negative test.[43,46,53] Low alpha-1 antitrypsin levels can also be seen in diseases associated with protein loss, such as via the kidneys or gastrointestinal tract.[43] A liver biopsy may be necessary to rule out other causes of liver disease.

All people with a first-degree relative with alpha-1 antitrypsin deficiency should be tested for the deficiency.[43] Heterozygosity for alpha-1 antitrypsin deficiency in the setting of a second cause of liver disease may aid in the progression to cirrhosis.[51] However, there is no therapy for the liver disease associated with alpha-1 antitrypsin deficiency other than transplant, and transplant cures the deficiency.[45,55]

Disease	Common Laboratory Abnormalities	Presentation of Disease
Alpha-1 antitrypsin deficiency	+AST/ALT	Early emphysema in smokers
	+Alkaline phosphatase	Jaundice or hepatitis in children
	Genotype analysis most commonly ZZ	Cirrhosis in children or adults
What test to order? Alpha-1 antitrypsin phenotype/genotype		

WILSON DISEASE

Wilson disease is caused by an autosomal recessive mutation in ATP7B, which helps transport copper into bile and bind copper to ceruloplasmin.[18,56–59] The incidence is as high as 1 per 30,000 and specifically the incidence in an Irish population was estimated at 17 per 1 million births with a prevalence of 3.6 per 1 million people.[18,58–61] The mutation in ATP7B leads to copper deposition in the liver as well as the brain, kidneys, and cornea leading to varying manifestations of the disease.[18,58]

Wilson disease most commonly presents between 5 years old and 40 years old, but it should be considered in patients with liver abnormalities between the ages of 3 and 55 years, especially if a patient has symptoms suggestive of Wilson disease.[58] Patients with Wilson disease can present with no symptoms and a mild increase in aminotransferase levels, hepatomegaly, neurologic symptoms, or acute liver failure with a Coombs-negative hemolytic anemia and acute kidney injury.[58,61–63] Many patients already have cirrhosis at presentation, which is usually present by the second decade of life.[57,58,62,63] A small percentage of patients present with hemolysis alone.[58,61] Similar to autoimmune hepatitis, patients with Wilson disease can have increased immunoglobulin and autoantibody levels.[58] A special population in which Wilson disease should be considered are patients with the diagnosis of autoimmune hepatitis who do not respond quickly to steroid treatment and any pediatric patient with the diagnosis of autoimmune hepatitis.[58,62]

Neurologic changes seen in Wilson disease usually present in the third decade of life, but small changes in childhood, such as handwriting or behavior, can be seen.[57,58,60] The neurologic findings in Wilson disease are usually parkinsonian characteristics, such as rigidity and dystonia, as well as dysarthria, and brain imaging can detect abnormalities in the basal ganglia, although this is not diagnostic.[58,60] Features on MRI brain most consistent with Wilson disease include signal changes in the

midbrain tectal plate; changes similar to those seen in central pontine myelinolysis; involvement of the basal ganglia, thalamus, and brainstem at the same time; as well as an image called the face of the giant panda.[64] Patients may also present with psychiatric disorders.[58,60] Patients with neurologic symptoms from Wilson disease usually first have liver disease and are often cirrhotic at the time of diagnosis.[60,65] Any patients with liver disease and neurologic or psychiatric findings should be tested for Wilson disease.[58]

Ceruloplasmin can be used as an initial screening examination for Wilson disease.[66] Ceruloplasmin level less than 20 mg/dL has a sensitivity of 95% and specificity of 84.5% for Wilson disease in children with increased aminotransferase levels.[66] Kayser-Fleischer rings, caused by copper deposition in the cornea and diagnosed by slit-lamp eye examinations, are seen in 44% to 62% of patients with hepatic Wilson disease and in about 85.5% to 95% of patients with neurologic Wilson disease.[57,58,60,67] If low ceruloplasmin level (<20 mg/dL), high 24-hour urine copper level (>40 μg), and Kayser-Fleischer rings are present, then the diagnosis of Wilson disease is confirmed.[57,58,65,66,68] If only 2 of the 3 tests are positive, then a liver biopsy for histology and quantification of copper level (>250 μg/g dry weight of liver) is necessary.[57,58,61] Genetic testing can be performed if patients have an intermediate copper quantification (50–250 μg/g) or if they have an increased urine copper level, Kayser-Fleischer rings, but do not meet the cutoff for copper quantification in the liver.[58]

Wilson disease can be difficult to diagnose. Kayser-Fleischer rings are not specific for Wilson disease because they can be seen in patients with chronic cholestasis from other forms of liver disease.[60,67] The copper quantification cutoff value of 250 μg/g dry weight of liver has a sensitivity 83.3% and a specificity of 98.6% for the diagnosis of Wilson disease.[69] When 75 μg/g dry weight of liver is used as a cutoff, the sensitivity is 96.5% with a decreased specificity of 95.4%.[69] Therefore a level of hepatic copper concentration between 50 and 250 μg/g dry weight of liver should lead to further investigation into the cause of liver disease and it does not rule out Wilson disease.[69] However, copper staining alone is not useful because it is variable in patients with Wilson disease and increased copper binding protein levels can also be seen in cholestasis from other causes.[60,69] Ceruloplasmin is also not a perfect test because it is an acute phase reactant and its levels can be increased in patients with high estrogen levels; for example, during pregnancy.[58,60–62,66,67] Ceruloplasmin level can also be low in patients who are losing proteins via the kidneys or intestines, with lack of production in end-stage liver disease, or with aceruloplasminemia.[58,60,61] Therefore, if ceruloplasmin level is increased but there is still a high suspicion for Wilson disease, a 24-hour urine copper test can aid in diagnosis.[60,66]

In patients who present with acute liver failure caused by Wilson disease, classic findings include Coombs-negative hemolytic anemia, rapidly progressive acute kidney injury, increased serum aminotransferase levels less than 2000 IU/L, and a normal or low alkaline phosphatase test.[58,60–62] Patients who present with acute liver failure usually already have cirrhosis at presentation.[60] The diagnosis of acute Wilson disease is critical because it does not respond to chelation or any medical therapy and thus urgent liver transplant is indicated and the only effective therapy.

A liver biopsy done in patients with Wilson disease can appear similar to biopsies from patients with NAFLD and can show signs of cholestasis, which does not distinguish it from other types of liver disease.[57,58,60,70] The liver biopsy can have iron overload caused by low ceruloplasmin level, which is important in the oxidation and transport of iron, hemolysis, inflammation, and cirrhosis.[15,70] A liver biopsy can help rule out other causes of liver disease.[60] In addition, an increased free serum copper

level (not bound to ceruloplasmin) may aid in the diagnosis, although it can be increased in other causes of acute liver failure, cholestatic liver disease, and in a copper overdose.[57,60–62]

Those patients with a first-degree relative with Wilson disease should be tested for the disease.[57,58] Genetic testing can be used as the primary means of diagnosis if the affected relative's genotype is known, because there are multiple mutations on ATP7B that have been identified and patients can be compound heterozygotes.[57,58,60,61] Genotype testing can help confirm the diagnosis in patients with other laboratory tests suggestive of Wilson disease, especially in populations in which certain mutations are more common.[60,65]

Disease	Common Laboratory Abnormalities	Presentation of Disease
Wilson disease	+AST/ALT	Hepatomegaly
	Low or normal alkaline phosphatase level	Cirrhosis
	Low ceruloplasmin level	Neurologic symptoms
	+24-h urine copper	Acute liver failure
	+Serum free copper	Kayser-Fleischer rings
	+Hemolysis work-up	
	Genetic analysis with ATP7B mutation	
What test to order? *ATP7B* genotype		

SUMMARY

Genetic causes of liver disease lead to a wide range of presentations, from mildly abnormal liver tests to acute liver failure. The most common cause of hereditary hemochromatosis is a mutation in the C282Y gene. If this genotype is absent, it is important to rule out other causes of liver disease because alcohol-related liver disease, NAFLD, viral hepatitis, and all causes of cirrhosis can present with blood tests consistent with iron overload.

Gilbert syndrome is a benign cause of indirect hyperbilirubinemia with no signs of hemolysis. PFIC can cause chronic cholestasis and patients usually present between the neonatal period and young adulthood. It is a rare disorder and other causes of cholestasis should be excluded first. Patients with PFIC1 and PFIC2 present with a normal GGT level despite cholestatic liver tests. BRIC is a benign cause of recurrent cholestasis that does not lead to chronic liver disease. Patients with BRIC have a normal GGT level during the episodes of cholestasis.

Patients with Wolman disease, the most severe form of LAL-D, present in childhood and adrenal calcifications are often seen on imaging. Liver biopsies in Wolman disease and CESD show microvesicular steatosis and cholesterol accumulation. The mutation that commonly causes liver disease in alpha-1 antitrypsin deficiency is PiZZ. Patients can present with increased aminotransferase levels, jaundice, and/or cirrhosis. Wilson disease can cause both neurologic disease and liver disease. Patients can present with a spectrum of liver disease from increased aminotransferase levels to fulminant liver failure. In children with abnormal liver tests and positive autoimmune hepatitis serologies, Wilson disease should be ruled out.

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