Key Concepts

- To know which liver diseases currently have genetic tests available for use in clinical practice
- To understand the role of genetic testing in the diagnosis of liver disease
- To understand the role of genetic testing in the prognosis of liver disease
- To understand the limitations of genetic testing in liver disease
- To develop an approach to the interpretation of genetic testing

Summary

Due to the tremendous progress in molecular genetics in both our understanding and its techniques, genetic testing for individuals is now available. In a number of liver disorders, genetic testing has been incorporated into routine clinical practice for prediction of predisposition to disease and prognosis. This review will summarize the currently available tests available in hemochromatosis, alpha-1-antitrypsin deficiency, Wilson Disease, autoimmune hepatitis, cholestasis, nonalcoholic fatty liver disease, and hepatitis C.

Hemochromatosis

In hereditary hemochromatosis, mutations of genes involved in iron metabolism lead to increased intestinal iron absorption and ultimately end organ damage including cirrhosis and hepatocellular carcinoma. For many years, hemochromatosis was known to have a genetic predisposition and linked to the HLA-A3 locus. Ultimately, a candidate gene study identified HLA-A (later renamed HFE). In the original description, a G to A missense mutation resulting in a cysteine to tyrosine substitution at amino acid 282 (C282Y) was homozygous in 83% of 178 patients in the discovery cohort. C282Y homozygotes make up approximately 80% of cases of hereditary hemochromatosis. Hereditary hemochromatosis is more common in Caucasians and the most commonly identified genetic disorder in Caucasians. In a study of 99,711 primary care patients in North America, racial and ethnic prevalences for C282Y homozygosity were 0.44% in whites, 0.11% in Native Americans, 0.027% in Hispanics, 0.014% in Blacks, 0.012% in Pacific Islanders, and 0.00004% in Asians. Two less common but regularly identified mutations in the HFE gene are histidine substitution for aspartate at amino acid position 63 (H63D) serine substitution for cysteine at amino acid position 65 (S65C). These mutations are generally not associated with iron overload unless a compound heterozygote with C282Y. A small proportion of patients with hereditary hemochromatosis have been identified to have mutations or absence of HFE, hemojelivin (HJV), hepcidin (HAMP), and transferrin receptor-2 (TFR-2) genes. Two forms of juvenile hereditary hemochromatosis are caused by mutations in HAMP and HJV. Testing for the HFE
mutations is broadly available and part of the accepted algorithm in the AASLD guideline for the evaluation of patients with elevated ferritin and transferrin saturation greater than 45%. [2] For a patient who is C282Y homozygous with elevated iron indices, the diagnosis of hemochromatosis is confirmed without liver biopsy. If other mutation patterns are determined, further evaluation including liver biopsy is required.

Clinical Scenarios

Question: is liver biopsy indicated in patients who are C282Y homozygous with elevated ferritin?

Patients who are C282Y homozygotes with elevated ferritin do not need a biopsy to initiate phlebotomy. The role of the biopsy here is to stage the disease and identify patients with cirrhosis. Patients with cirrhosis require closer monitoring including hepatocellular carcinoma surveillance. The AASLD guideline states, “Liver biopsy is recommended to stage the degree of liver disease in C282Y homozygotes or compound heterozygotes if liver enzymes (ALT, AST) are elevated or if ferritin is <1000 μg/L.” The presence of cirrhosis has been reported at 20–45% in patients with ferritin greater than 1000 μg/L and rarely present with lower ferritin values. These criteria may not apply if patients have excessive alcohol use or another co-existing form of liver disease.

A 48 year old man was recently diagnosed with hereditary hemochromatosis. HFE mutation analysis revealed he is a C282Y homozygote. His younger brother was also found to be C282Y homozygous but was informed that he did not need phlebotomy, and the patient asked why this might be the case. The patient also has three younger children ranging from ages 18 to 26. He was recommended to have them tested but is reticent to do this.

The AASLD guideline states, “We recommend screening (iron studies and HFE mutation analysis) of first-degree relatives of patients with HFE-related HH to detect early disease and prevent complications.” For the younger brother, phlebotomy would not be indicated if the ferritin is normal. Presumably, the brother has a normal ferritin and should receive annual testing. In the case of children, testing the other parent would guide the risk for the children, who are heterozygotes unless the other parent also carries a HFE mutation.

A 60-year-old woman was referred after she was found to be a C282Y/H63D compound heterozygote. She had presented with AST 30 U/L and ALT 72 U/L. Ferritin was 350 μg/L and transferrin saturation was 35%. Liver biopsy revealed minimal iron in Kupffer cells and the dominant feature was nonalcoholic steatohepatitis. The patient asks if phlebotomy is recommended.

This case highlights that not all patients with these common HFE mutations develop hemochromatosis. In cases of family screening, an individual may be identified at an early age before they develop hemochromatosis. Approximately 70% of C282Y homozygotes and less than 10% of C282Y heterozygotes develop severe iron overload. To categorize these patients, the European Association for the Study of Liver Diseases described the following three stages [5]:

Stage 1: genetic disorder present but no increase in iron stores; “genetic susceptibility.”
Stage 2: genetic disorder present and phenotypic evidence of iron overload but no organ damage.
Stage 3: genetic disorder with iron overload and iron deposition with tissue and organ damage.

Alpha-1-antitrypsin Deficiency

Alpha-1-antitrypsin (A1AT) is a serine protease inhibitor, and deficiency causes emphysema that develops in middle age, but the second most common affected organ is the liver. In children, cholestatic jaundice and hepatitis can develop soon after birth, while others present later in childhood with abnormal liver enzymes and progressive liver disease. Adults may present with mild asymptomatic liver enzymes through cirrhosis and hepatocellular carcinoma. The disorder has an autosomal recessive inheritance pattern with co-dominant expression given that each allele contributes 50% of the total circulating enzyme. The A1AT gene is located on the long arm of chromosome 14, and more than 100 alleles have been identified. The M allele is the most common for the A1AT gene and is associated with normal levels of A1AT. The alleles most commonly associated with severe liver disease are S and Z, leading to expression of 50 to 60% and 10 to 20% of A1AT, respectively. More rare alleles associated with liver disease include M (Malton) and M (Duarte). [6] Approximately 2% of the US population is heterozygous for the Z allele while 0.05% is homozygous. Racial and ethnic variation has been reported with the condition rare in Asians, uncommon in African Americans (2.6 per 1,000), and more common in Latinos (9.1 per 1,000) and white Americans (14.0 per 1,000). [7] It has been reported that less than 10% of patients with A1AT deficiency have been diagnosed with delays of many years in the diagnosis. Despite the presentation in infancy, newborns are not screened for A1AT deficiency in North America. Testing for A1AT deficiency is recommended in patients with liver disease of unexplained.
etiology and those with a family history of A1AT deficiency. A family history of lung and or liver disease should raise increased suspicion for A1AT. [8]

**Clinical Scenario**

A 70 year old man is admitted to hospital for the treatment of pneumonia. Consult is obtained for evaluation of abnormal liver enzymes. His results include AST 72 U/L, ALT 81 U/L with normal alkaline phosphatase and bilirubin. Serologic evaluation was completed with no obvious cause of liver disease. His A1AT plasma concentration was normal at 120 mg/dl. Question: is genotype testing necessary to rule out A1AT deficiency?

Testing options for A1AT deficiency include measurement of serum or plasma levels of A1AT, and genotype analysis. The A1AT concentration is a common initial test, and levels less than 50% of the lower limit of normal are indicative of this diagnosis. A1AT is an acute phase reactant and could be inflated in the setting of inflammation such as the case of the hospitalized patient. As a result, the A1AT concentration should not be used to rule out the diagnosis in this situation. Genotype analysis identifies the individual variants to be identified and determines the phenotype. The common deficiency phenotypes include PiZZ, PiSZ, and PiSS. Common carrier phenotypes include PiMS and PIMZ. The major societies do not currently have guidelines with an algorithm for testing for A1AT deficiency, but the combination of the serum level of A1AT and the genotype are generally recommended.[8]

**Wilson Disease**

Wilson disease was initially described in 1912 with the combination of neurologic disorders and liver disease. During the next several decades, the role of copper accumulation in affected individuals was understood, and the inheritance pattern was determined to be autosomal recessive. The prevalence of Wilson Disease is 30 persons per million worldwide. Wilson Disease usually presents in children and younger adults, but all age groups are recommended for diagnostic consideration. The spectrum of liver disease includes presentations with acute liver failure through more chronic liver disease and cirrhosis.[9] In 1993, the ATP7B gene was identified in patients with Wilson Disease on chromosome 13. ATP7B encodes an ATPase that is a copper transporter expressed within hepatocytes. The reduced ATP7B protein leads to reduced excretion of copper from hepatocytes with resulting injury.[10] The diagnosis of Wilson Disease has traditionally been determined through the identification of clinical features and laboratory tests. The classic presentation includes the presence of Kayser-Fleischer rings, ceruloplasmin less than 20 mg/dl and 24-hour urine copper greater than 40 mcg.

**Clinical Scenarios**

A 20 year old woman presents with AST 62 U/L and ALT 91 U/L. These tests have been abnormal on repeat testing over the last 6 months. She reports liver disease in her maternal grandfather with unclear history on the role of alcohol. Serologic testing for viral hepatitis and autoimmune hepatitis were negative. The patient has no risk factors for fatty liver disease. Ceruloplasmin was 15 mg/dl, but Kayser-Fleischer rings were absent on slit lamp examination, and 24-hour urine copper was 30 mcg. Liver biopsy was performed and revealed copper quantification of 100 mcg/kg dry weight. Does the patient have Wilson Disease?

This patient highlights that many patients do not demonstrate classic features. If one or two of these diagnostic criteria are present, liver biopsy can be performed, and the diagnosis is established if copper quantification is greater than 250 mcg/kg dry weight. In cases of family screening or in younger people, the patient may be at an early stage of the disorder and not have achieved significant copper accumulation. In these cases, genetic testing can provide more insight into the diagnosis. ATPB7 DNA sequencing is available through commercial reference laboratories. More than 500 mutations have been reported in Wilson Disease with regional patterns noted around the world, and most patients carry two different mutations and are therefore compound heterozygotes. The possibility of an unidentified mutation must remain a consideration for the patient with negative mutational analysis.[11]

A 24 year old man presents for evaluation. His younger sister presented with fulminant Wilson Disease and required liver transplantation. The patient inquires about the role of genetic testing for him.

Genetic testing should be considered in the evaluation of a first degree relative with Wilson Disease. If the molecular sequence for the index patient is known, genetic testing is the most efficient strategy for screening relatives and is recommended by the AASLD guideline.[9]

**Autoimmune Hepatitis**

Autoimmune hepatitis (AIH) was initially described in 1950, and the clinical features associated with the condition...
include chronic inflammation, interface hepatitis, hypergammaglobulinemia, and the production of autoantibodies.[12] The disorder is more common in females than males and can present at any age. The most common autoantibodies in type 1 AIH are antinuclear antibodies and anti-smooth muscle antibody. Type 2 AIH is less common, usually presents in young girls, and the anti-liver kidney microsomal antibody is produced. Genetic studies have demonstrated that susceptibility to type 1 AIH is mediated by HLA alleles. Studies in Europe and North America have revealed that HLA DRB1*0301 and DRB1*0401-DQA1*03-DQB1*0301 are associated with increased risk, while HLA DRB1*1501-DQA1*0102-DQB1*0602 is observed at lower frequency in patients with AIH. Fewer studies have been conducted in patients with type 2 AIH, and there is suggestion of association with HLA DRB1*07 and HLA DQB*0201.[13] For patients receiving treatment with azathioprine or 6-mercaptopurine, testing for a polymorphism in thiopeptide S-methyltransferase or TPMT gene can be considered. Azathioprine is converted to 6-mercaptopurine, and then 6-mercaptopurine may be inactivated (by TPMT to 6-methylmercaptopurine or by xanthine oxidase to 6-thiouric acid) or converted by a multiple step enzymatic pathway to the active metabolites, the 6-thioguanine nucleotides. The distribution of enzyme activity of TPMT is trimodal: homozygous low activity occurs (frequency 0.3%), heterozygous or intermediate activity (frequency 11%); and homozygous high or normal activity (frequency of 89%).[14] For TPMT homozygotes with low enzyme activity, azathioprine and 6-mercaptopurine use could result in severe hematopoietic toxicity. TPMT heterozygotes have risk of leukopenia and may require reduced dosing of azathioprine or 6-mercaptopurine.[13] Although knowledge of the TPMT genotype might guide selection of medication and dosing in some patients, current guidelines do not mandate testing prior to the initiation of medication. TPMT genotype does not predict toxicity for all patients, and therefore standard hematologic monitoring is still recommended in patients with normal TPMT genotype.

**Cholestasis**

Progressive familial intrahepatic cholestasis (PFIC) includes three disorders with defective secretion of bile acids leading to cholestasis. These disorders have been observed worldwide in both genders. The true incidence is unknown but estimates suggest between 1/50,000 and 1/100,000 live births. PFIC1 and PFIC2 typically present in the first few months of life with pruritus and jaundice and a progressive leading to fibrosis and portal hypertension. PFIC2 can lead to hepatocellular carcinoma within the first year of life. PFIC3 has similar presentation but may occur later in infancy and even as late as young adulthood. The molecular mechanisms of all three variants have been determined (see Table 1).

PFIC1 is caused by a defect in the ATP8B1 gene encoding the FIC1 protein and leading to impaired bile salt secretion. PFIC2 has a similar mechanism, but the defect is in the ABCB11 gene encoding bile salt export pump (BSEP) protein. In PFIC3, defects in the ABCB4 gene encoding multidrug resistance 3 protein (MDR3) disrupt biliary phospholipid secretion.[15] The diagnosis of PFIC should be considered in all infants and children presenting with pruritus and jaundice with elevation in conjugated bilirubin. Serum gamma-glutamyltransferase (GGT) is normal in PFIC1 and PFIC2 but is elevated in PFIC3. Other disorders presenting with cholestasis should be considered. The differential diagnosis includes extrahepatic bile duct obstruction, biliary atresia, Alagille syndrome, PSC, A1AT deficiency, and cystic fibrosis. The diagnostic evaluation will include appropriate serologic testing and liver ultrasound. Cholangiography may also be indicated, and biliary lipid composition can then be performed. Liver biopsy is also often required, and immunostaining for BSEP and MDR3 can suggest PFIC disorders. Molecular diagnostic testing for these disorders is available through reference laboratories for PCR-based sequencing of the ATP8B1, ABCB11 or ABCB4 genes. In addition to PFIC, mutations in ATP8B1, ABCB11 and ABCB4 have been observed in benign recurrent intrahepatic cholestasis (BRIC).[16, 17] BRIC is similar to PFIC except with intermittent episodes of cholestasis, pruritus and diarrhea.

**Nonalcoholic Fatty Liver Disease**

Nonalcoholic fatty liver disease (NAFLD) continues to emerge as a major cause of liver disease throughout the world, and research into the pathophysiology has revealed genetic predisposition. In 2008, a genome wide association study revealed that allele in patatin-like phospholipase domain containing 3 or PNPLA3 (rs738409) was strongly associated with increased hepatic fat content and hepatic

### Table 1. Progressive familial intrahepatic cholestasis characteristics

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome</th>
<th>Inheritance pattern</th>
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<tbody>
<tr>
<td>ATP8B1</td>
<td>18q21-22</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>ABCB11</td>
<td>2q24</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>ABCB4</td>
<td>7q21</td>
<td>Autosomal recessive</td>
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### Table 2. Genetic predisposition to type 1 AIH

<table>
<thead>
<tr>
<th>Gene</th>
<th>Inheritance pattern</th>
</tr>
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<tbody>
<tr>
<td>HLA DRB1<em>0301-DQA1</em>03-DQB1*0301</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>HLA DRB1<em>1501-DQA1</em>0102-DQB1*0602</td>
<td>Autosomal recessive</td>
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*American Association for the Study of Liver Diseases*
inflammation. The allele was most common in Latinos. Another allele of PNPLA3 (rs6006460) was associated with lower hepatic fat content in African Americans.[18] A subsequent meta-analysis in 2011 included 16 studies and also demonstrated that patients homozygous (GG) at rs73840 had greater liver fat accumulation, greater risk of higher necroinflammatory scores, and greater risk of fibrosis.[19] PNPLA3 therefore does predict risk and the course of NAFLD, but there are no current recommendations for testing as part of routine clinical care. Studies of the etiology and natural history of NAFLD should include assessment for the contribution of PNPLA3.

Hepatitis C

In 2009, the initial report from a GWAS emerged that HCV treatment outcomes were predicted by the polymorphism rs12979860 on chromosome 19 near the IL28B gene.[20] The less favorable genotype was more common in African Americans and explained much of the racial variation observed in HCV treatment. Subsequent studies demonstrated another polymorphism (rs8099917) in close proximity and in strong linkage disequilibrium.[21, 22] The mechanism behind this association was not immediately clear, but a recent report suggested that a frame shift variant upstream of IL28B leads to the gene IFNL4, which encodes the interferon-λ4 protein. These authors found that overexpression of IFNL4 in a hepatoma cell line resulted in STAT1 and STAT2 phosphorylation and the expression of interferon-stimulated genes.[23] These studies were conducted among patients who were treated with peginterferon-α and ribavirin, and they demonstrated that patients with the favorable IL28B genotype achieved SVR rates more than twice the rates of patients with the less favorable genotype.[20] The IL28B genotype is the strongest predictor of SVR in patients treated with peginterferon-α and ribavirin and can be used to guide patients considering HCV treatment with this regimen. However, the clinical role for the IL28B genotype was impacted by the development of direct acting antivirals released in 2011 in the United States and Europe. The improved response rates with boceprevir and telaprevir raised further questions about the role or value of the IL28B genotype in these regimens. An analysis of the boceprevir program demonstrated that patients with the favorable IL28B genotype continue to have the highest SVR rates, but all genotypes benefit from the addition of the protease inhibitor.[24] Patients with the favorable IL28B genotype are more likely to qualify for shortened duration of treatment in the response guided therapy algorithm.

<table>
<thead>
<tr>
<th>Racial/ethnic group</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
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<tbody>
<tr>
<td>Caucasian</td>
<td>69%</td>
<td>33%</td>
<td>27%</td>
</tr>
<tr>
<td>Hispanic</td>
<td>56%</td>
<td>38%</td>
<td>27%</td>
</tr>
<tr>
<td>African American</td>
<td>48%</td>
<td>15%</td>
<td>13%</td>
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The 2011 update of the AASLD guideline makes the following statement about IL28B genotype testing: IL28B genotype is a robust pretreatment predictor of SVR to peginterferon alfa and ribavirin as well as to protease inhibitor triple therapy in patients with genotype 1 chronic hepatitis C virus infection. Testing may be considered when the patient or provider wish additional information on the probability of treatment response or on the probable treatment duration needed.[25]

Clinical Scenarios

A 50 year old Caucasian woman presents with a recent diagnosis of HCV. She has genotype 1a infection and early stage fibrosis. She has no significant comorbidities and is considered a good candidate for treatment. She lives in country X where standard of care for HCV treatment is peginterferon-α and ribavirin. Should she get IL28B genotype testing?

In general, diagnostic testing should be used if it helps to guide care. In this case, the intention to treat analysis from the IDEAL trial of treatment naïve patients would tell us that the likelihood of SVR is 69% if this patient has the favorable genotype or 27-33% if the less favorable genotype (see Table 2).[26] If this knowledge of the likelihood of response would impact the decision of the patient to accept treatment, the IL28B genotype should be performed.

A 50 year old Caucasian woman presents with a recent diagnosis of HCV. She has genotype 1a infection and early stage fibrosis. She has no significant comorbidities and is considered a good candidate for treatment. She lives in country Y where standard of care for HCV treatment is peginterferon-α, ribavirin and either boceprevir or telaprevir. Should she get IL28B genotype testing?

The results from the boceprevir program have been published and will therefore be highlighted here (see Table 3). Using the polymorphism rs12979860, the likelihood of SVR was 82% for CC genotype patients in the response guided therapy arm for naïve patients compared to 65% for genotype CT and 55% for genotype TT. Some patients might consider 55–66% a reasonable chance and proceed
with treatment. Other patients might decline therapy and await new treatments given the early stage of fibrosis. That opinion may also change if the patient had cirrhosis. The SVR rate is lower in patients with cirrhosis, but the desire to proceed with treatment may be greater for that patient concerned about the risk of progression to cirrhosis. Another factor that may guide patient decisions is the likelihood of shortened duration of treatment in the response guided algorithm. Patients with the favorable IL28B genotype were much more likely to qualify for the shortened duration of treatment with boceprevir (78%) and telaprevir (89%). The likelihood of 24-28 weeks of therapy versus 48 weeks might guide patient decisions. These decisions will take into account the predictors of response for that individual patient and their patient preferences.

A 50 year old Caucasian woman presents with a recent diagnosis of HCV. She has genotype 1a infection and early stage fibrosis. She has no significant comorbidities and is considered a good candidate for treatment. It is 2015, and she lives in country Z where standard of care for HCV treatment is an all oral regimen of multiple direct acting antivirals. Should she get IL28B genotype testing?

The answer to this question is currently unknown and will be an important topic to understand as new treatments emerge. Early indications suggest that the more potent regimens overcome the impact of the IL28B genotype, but this will need to be explored for each regimen.

References


