Hereditary Hemochromatosis

Impact of Molecular and Iron-Based Testing on the Diagnosis, Treatment, and Prevention of a Common, Chronic Disease

Richard D. Press, MD, PhD

Objective.—To review the current state-of-the-art regarding the role of iron- and DNA-based testing on the detection, treatment, and prevention of hereditary hemochromatosis (HH), the most common single-gene disorder in white people.

Sources.—Review of the medical literature, with particular emphasis on recent reports of the impact of DNA-based testing on the detection of symptomatic and presymptomatic patients with HH.

Conclusions.—Hereditary hemochromatosis, a common autosomal recessive iron overload disorder (with a population prevalence of 0.3%–0.8%), is a common cause of preventable liver, heart, joint, and endocrine disease. Since the associated clinical signs and symptoms are nonspecific, an accurate HH diagnosis demands both a high index of suspicion and the direct laboratory demonstration of elevated iron parameters. The substantial public health burden of HH as a common, deadly, detectable, and treatable chronic disease has led the College of American Pathologists to recommend that “systematic screening for hemochromatosis is warranted for all persons over the age of 20 years.” The recent discovery that most HH cases are the result of a single well-conserved homozygous missense mutation (C282Y) within a novel transferrin-receptor binding protein (HFE) has given rise to diagnostic clinical tests for the DNA-based detection of this pathologic mutation. This direct HFE mutation test can now be used not only to confirm the diagnosis of HH in those with symptomatic disease, but also, perhaps more importantly, to detect those with presymptomatic iron overload in whom future disease manifestations may be prevented (with phlebotomy therapy).

(Arch Pathol Lab Med. 1999;123:1053–1059)

Hereditary hemochromatosis (HH), a common autosomal recessive disorder of iron metabolism, is characterized by excessive absorption of dietary iron and its progressive, toxic accumulation in numerous systemic organs. Despite the perception of most physicians that HH is yet another rare, untreatable genetic disease, it is, in fact, both extremely common, affecting approximately 1 in every 200 whites, and, when detected early, very treatable. Although both affected individuals and their at-risk relatives can be readily identified using a variety of diagnostic laboratory tests, HH’s nonspecific clinical symptoms, long presymptomatic phase, and relative under awareness within the health care community contribute to the many misdiagnosed or undiagnosed cases. We have recently learned that most HH cases are caused by a single homozygous point mutation (C282Y) in the newly described transferrin-receptor binding protein HFE. Since the presence of this common mutation can be directly assessed in DNA from either symptomatic or presymptomatic patients, HH represents perhaps the best present-day example of the clinical contributions of molecular diagnostic testing to the treatment and prevention of an inherited disease, which by most criteria constitutes a true public health threat.

PATHOPHYSIOLOGY

The predominant pathologic defect in HH is an excess absorption of dietary iron. In contrast to the 1 to 2 mg of iron absorbed by the normal individual (and balanced by equivalent losses), HH homozygotes absorb approximately 3 to 4 mg per day. Since the body has no means of disposing of this 0.4 to 1.0 g per year of excess iron, it ultimately deposits within parenchymal cells of the liver (predominantly), pancreas, pituitary, synovium, heart, and elsewhere. After saturating intracellular ferritin stores, this excess free iron participates in intracellular redox reactions, ultimately contributing to the generation of the toxic reactive oxygen species that cause cell damage and/or death. Because the buildup of iron stores to organ-damaging levels requires several decades (but is extremely heterogeneous), the typical symptomatic HH patient often comes to clinical attention in middle-age (between ages 30–50 years) with one or several clinical features, including hepatic dysfunction, diabetes, hypogonadism, hyperpigmentation, arthritis, cardiomyopathy, and/or fatigue.1 Death in advanced cases is often from cirrhosis, diabetes, cardiomyopathy, or hepatocellular carcinoma.2,3 However, since a variety of additional variables significantly affect iron flux (diet, sex, other genes), the disease severity and age of presentation are highly variable. In particular, because women have mechanisms of iron loss not typical of...
men (menstruation and pregnancy), the case ratio is biased toward men (male:female ratio of ~1.2-1.8:1), and men typically present with earlier and more severe disease.

**PREVALENCE**

Hemochromatosis is by far the most common classically inherited disease in America. Screening iron studies on healthy control individuals have estimated that HH affects approximately 1 in every 125 to 333 individuals in the normal populations of the United States, Northern Europe, and Australia. This 0.3% to 0.8% population prevalence makes HH much more prevalent than other "common" (but better known) inherited conditions, such as cystic fibrosis, sickle cell anemia, phenylketonuria, α1-antitrypsin deficiency, and Tay-Sachs disease. A significant fraction of the white population (~12%-15%) thus carries at least one mutant hemochromatosis gene that, by itself, can be associated with mild or moderate iron overload and carries a 3% to 4% risk of producing a homozygous child. Although this disease is predominantly associated with Northern European ancestry, its population prevalence may be similarly high in Hispanics, and it is also present, albeit at lower rates, in African Americans (0.03%-0.09%), Southern Europeans, Ashkenazi Jews (0.02%), Chinese, and aboriginal Australians. In comparison, population prevalence estimates based on autopsy studies or death certificate reviews range from 0.03% to 0.2%, further confirming that this disease is both significantly underrecognized by the health care community and its clinical expressivity (penetrance) is less than 100%.

**THERAPY**

Yet another unique attribute of HH among inherited diseases is the wide availability of an effective, inexpensive therapy—therapeutic phlebotomy. Once identified, patients with HH should undergo phlebotomy once or twice per week (each 500-mL unit removing ~250 mg of iron). Weekly phlebotomies should continue until iron stores have been depleted as judged by serum ferritin (SF) levels below 20 to 50 µg/L (or hemoglobin levels less than 110 g/L in patients without prior anemia). Following this initial "deironing" phase, maintenance phlebotomies will be required in perpetuity (typically 3 to 6 times per year) to always maintain the SF level below 50 µg/L. An added benefit of phlebotomy is a precise quantitation of excess iron loads (by tallying cumulative blood loss). The demonstration of the removal of more than 5 g of iron (without inducing anemia) provides definitive proof of an HH diagnosis (regardless of genotype) and suggests the initiation of family studies. Those patients identified in the early presymptomatic stage of disease (within families or by screening iron or genotype studies) should probably also undergo phlebotomy if their SF levels exceed normal limits (200 µg/L in premenopausal women, 300-400 µg/L in men). If phlebotomy is initiated before the onset of diabetes or cirrhosis, life expectancy returns to normal and the patient is "cured." Phlebotomy therapy of patients with cirrhosis or diabetes, although not curative, provides a significant survival advantage. In addition, therapy is typically also associated with a resolution of some clinical symptoms (weakness, hepatomegaly, cardiomyopathy, hyperpigmentation) and a marked improvement in quality of life. Since other clinical symptoms (cirrhosis, diabetes, arthropathy, hypogonadism) respond less frequently to treatment, the identification of predisposed individuals before the onset of clinical symptoms should be the primary goal for the management of this preventable disease.

**Genetics of HH**

Hereditary hemochromatosis has long been recognized as a classic autosomal recessive disorder with sex-dependent penetrance (men more than women) linked to the HLA locus on chromosome 6. Before the discovery of the causative gene defect, the linkage of this disease to a specific HLA serotype (HLA-A3) was often used to predict affected family members. However, since only approximately 70% of patients with HH carry an A3 antigen, and A3 is a common allele (present in ~28% of the general white population), the positive predictive value of HLA-A3 serotyping as a front-line diagnostic test is extremely poor (only ~1%). An intensive search for the causative gene on the short arm of chromosome 6 ultimately led, in 1996, to the discovery of the HH-associated disease gene, initially named HLA-H and subsequently renamed HFE. Sequence analysis of this 343 amino acid integral membrane protein revealed it to be a member of the major histocompatibility class I family with 2 extracellular functional domains—a peptide-binding region (with α1 and α2 loops), and a β2-microglobulin-binding immunoglobulin-like domain (α3 loop). The HFE protein, although ubiquitously expressed in many tissue types (albeit at low levels), appears localized in an intracellular and perinuclear distribution within small intestinal crypt epithelial cells (the putative site of iron absorption) compared with its membrane localization in other gastrointestinal cell types. More recent structural and functional analyses have revealed that the HFE protein physically interacts with and colocalizes with the transferrin receptor and in so doing decreases its affinity for iron-bound transferrin. HFE is thus thought to be a negative regulator of transferrin receptor-mediated uptake or handling of transferrin-bound iron. In the latest model of HH pathophysiology, when HFE function is lost (as in patients with HH), this negative feedback loop is abrogated, and unregulated constitutive cellular iron uptake proceeds. The severe phenotypic iron overload in "knockout" mice with a homozygous disruption of the HFE gene serves as definitive proof both that HFE is the true hemochromatosis gene and that the HH phenotype is due to loss of HFE function (and not due to changes in either HFE's function or its subcellular location).

**HFE MUTATIONS IN HH**

The mechanism for the loss of HFE function in most patients with HH is a single well-conserved point mutation at amino acid 282 (nucleotide 845 G → A). This C282Y mutation disrupts a disulphide bond in the α1 loop, abrogates HFE binding to β2-microglobulin, and prevents cell surface expression of HFE. The disease-associated C282Y substitution, as was predicted from the genetics of HH, is a true loss of function mutation since 282Y HFE, unlike its wild-type homologue, does not associate with the transferrin receptor and does not down-regulate transferrin receptor's affinity for iron-bound transferrin.
PREVALENCE OF HFE C282Y IN PATIENTS WITH HH AND CONTROLS

A number of studies of patients with well-characterized HH from around the world have shown that the pathogenic genetic alteration in 70% to 100% of patients with HH is a homozygous C282Y point mutation. Of the 960 patients with HH summarized in Table 1 (from 9 different studies), an average of 83% were C282Y homozygotes. The true sensitivity of the C282Y homozygous mutation for predicting HH is probably closer to 90%, since approximately half of the nonhomozygous patients with HH in these initial studies have recently been shown to have previously undetected secondary iron overload disorders.29 This high degree of genetic homogeneity is most likely the result of a relatively new iron-loading founder mutation (C282Y) that, because of its associated survival advantage (particularly in reproductive-age women), has persisted through the generations. In comparison, as predicted from screening iron studies in normal populations, the C282Y HFE mutation is present in heterozygous form in approximately 10% to 15% of any of several white populations so far analyzed (Table 2). Of the 2311 pooled normal individuals genotyped in Table 2 (within 8 separate studies), the allele frequency for the C282Y mutation averaged 6.6%, the heterozygote frequency averaged 12.3%, and the homozygote frequency averaged 0.4%. These population-based C282Y genotype frequencies agree closely with estimates of the high population prevalence of HH from screening iron studies, serving as epidemiologic confirmation that this is the predominant genotype in most of those with primary iron overload.

Although most patients with HH will be found to have 2 mutant HFE C282Y alleles, 10% to 20% will not. In some studies, a significant minority of these nonhomozygotes (~3%–5% of total patients with HH) had, in addition to a
heterozygous C282Y mutation, a second minor alteration (H63D) on the other HFE allele in which amino acid 63 (nucleotide 187) was an aspartic acid instead of a histidine (Tables 1 and 2). Although this H63D change is an extremely common polymorphism in normal whites (heterozygotes representing ~25% of the normal population), when it is inherited together with the major C282Y mutation, the resulting compound heterozygous genotype has been found to be significantly enriched in those with HH compared with normal controls.\(^{30,31}\) However, since approximately 2% of the normal population carries this compound heterozygous genotype, most of whom are free of iron overload, the clinical penetrance of this compound heterozygous genotype is clearly very low (~0.5% to 1%).\(^{30,31}\) Whether those with a homozygous H63D mutation may also be at increased risk for HH remains unclear; if so, given the 2% population prevalence for H63D homozygosity, its relative clinical penetrance would be extremely low (less than ~0.2%). Since most of those with the H63D polymorphism will never develop iron overload, to avoid potential stigmatization or genetic discrimination, the clinical utilization of H63D genotype data should be strictly reserved for evaluations of those with clear phenotypic iron overload in the absence of C282Y homozygosity.

**DIAGNOSIS OF HH**

**Laboratory Parameters of Phenotypic Iron Overload**

Because HH is a common but underrecognized disorder with heterogeneous and nonspecific clinical presentations, the most important factor in its diagnosis is maintaining physician awareness. Even when suspected, primary HH must always be distinguished from other causes of iron overload, including dyserythropoietic anemias, African iron overload, transfusions, porphyria cutanea tarda, and medicinal iron use.\(^7\) The predominant HH clinical findings of fatigue and weakness, liver disease, arthropathy, impotence, amenorrhea, diabetes, hyperpigmentation, and cardiac disease are thus often attributed to non–iron-related causes. Approximately 25% to 50% of obligate HH homozygotes (within HH families) have no clinical symptoms (yet),\(^{32,33}\) and only a few homozygotes have the classic diagnostic triad of liver disease, diabetes, and skin bronzing.\(^{5,18}\) Even among those with end-stage liver disease undergoing liver transplantation, up to 35% of those with HH (by hepatic iron studies) lacked a correct pretransplant diagnosis.\(^{34}\) The effectiveness of early phlebotomy therapy in restoring normal life expectancy\(^{16,17}\) further worsens the tragedy of these delayed or missed diagnoses.

Once an iron overload disorder is suspected, the clinical diagnosis of HH requires the demonstration, in tissue and/or blood, of excess stores of iron. A recommended diagnostic algorithm is shown in the Figure. The consensus first-line screening test to assess systemic iron overload is quantitation of the percentage of transferrin saturation (TS) (serum iron divided by total iron-binding capacity).\(^{17}\) In HH homozygotes, TS is typically the first laboratory parameter to become abnormal (often by adolescence),\(^1\) and it is elevated (above 45%) in more than 95% of HH homozygotes.\(^{13,33}\) The true diagnostic sensitivity for TS depends on many factors, most importantly sex (TS being lower in females) and the chosen diagnostic cutoff level (higher cutoffs having lower sensitivities). Most studies, however, have used TS cutoffs of 45% to 62% to detect 86% to 98% of HH homozygotes with specificities of 93% to 99%.\(^7,8,33,35\) Because TS levels can be affected by diurnal variation, dietary factors, laboratory imprecision, or concomitant disease states (inflammation or hepatitis), an initially elevated TS test result should be followed up with a repeat fasting TS measurement.\(^1,7\) The College of American Pathologists has recently recommended a TS cutoff of more than 60% (on two occasions) as the initial diagnostic criteria for HH.\(^7\)

A repeatedly elevated TS level in the absence of other plausible causes requires additional laboratory evaluations to estimate body iron stores. In particular, an SF level above normal limits (200 µg/L for premenopausal women; 400 µg/L for men) in the presence of a persistent TS level elevation (but without evidence of inflammation, cancer, or hepatitis) implies, by definition, the presence of primary iron overload.\(^1,7\) Although the progression of iron overload varies widely in patients with HH, most will show elevated SF levels by young adulthood.\(^1,10\) In contrast, since the failure to demonstrate an elevated SF level does not rule out HH (particularly in younger individuals and females), these subjects should be monitored by repeat SF measures at least every other year.\(^7\)

Before the discovery of the HFE gene, the consensus next step in the HH diagnostic algorithm (after TS and SF determinations) would be a liver biopsy with histochemical and biochemical iron determinations. The traditional gold standard HH diagnostic test has thus been a liver biopsy specimen showing abundant parenchymal cell iron after Perls’ staining (grade 3 to 4; typically heavier in portal areas). In the presence of stainable hepatocellular iron, the hepatic iron should be directly quantitated, typically by atomic absorption spectrophotometry, and in HH will almost always be greater than 80 µmol per gram of dry weight.\(^7\) Furthermore, an age-weighted hepatic iron index (HII) (iron concentration divided by age) has been shown to be the best quantitative discriminator of homozygous HH from other iron-loading liver diseases (alcoholism or hepatitis). Although an HII greater than 1.9 µmol/g per year was once thought to be pathognomonic for HH,\(^7,26\) we have recently shown that the HII has poor diagnostic specificity in patients with cirrhosis (23% positive predictive value).\(^37\) In comparison, in the noncirrhotic liver, an HII greater than 1.9 both confirms the diagnosis of HH and predicts a normal posttherapy life expectancy.

**Direct DNA Analysis for the C282Y HFE Mutation**

The major distinction between the diagnostic algorithm shown in the Figure and those from the pre-HFE era is the recent availability of a direct DNA test for the disease-causing C282Y mutation. The presence of this single well-conserved point mutation in most patients with HH has led many molecular pathology and/or genetics laboratories to create polymerase chain reaction–based diagnostic tests to assess its presence. Although the collective clinical experience with HH DNA testing is rather short, the availability of this test has already made a positive impact on both increasing the detection rate for this common disease and, perhaps more importantly, shifting the diagnostic strategy toward identification of presymptomatic patients who have not yet accumulated sufficient iron to cause organ damage. In particular, the specific clinical scenarios in which HFE direct mutation testing will have an immediate impact include the following:
Diagnostic testing algorithm for hemochromatosis.

1. The Direct Diagnosis of HH in Those With Elevated Serum Iron Stores (TS and/or SF).—Our recommended diagnostic algorithm (Figure) suggests that liver biopsy may no longer always be required for an accurate HH diagnosis. In particular, in those iron-loaded patients with a minimal risk of cirrhosis and thus excellent prognostic features (those with SF levels less than 1000 μg/L and a normal aspartate aminotransferase level), the demonstration of C282Y homozygosity confirms the diagnosis of HH without the risks of liver biopsy. Candidates for direct DNA testing should then include patients with elevated TS levels with or without elevated SF levels—the latter group including the presymptomatic HH homozygotes in whom monitoring and early therapy will likely prevent disease manifestations. In our laboratory, for example, of the 1444 patients undergoing HH DNA tests during the past 2.5 years for the evaluation of iron overload, a large fraction (17%; 238 individuals) were found to be C282Y homozygotes. The failure to detect 2 C282Y alleles does not, of course, rule out HH (diagnostic sensitivity of ~90%), and liver biopsy may then be required for a definitive diagnosis.

2. The Direct Diagnosis of HH in Those With Non-diagnostic Iron-Loaded Liver Biopsy Specimens.—In particular, we have recently found that cirrhosis, per se, often elevates hepatic iron levels into the HH range (HII = 2–3 μmol/g per year) and, conversely, that some proven HH homozygotes (mainly females) have HII’s below 1.9. In these patients and those with hepatic iron overload of other uncertain origins, the demonstration of C282Y homozygosity can be diagnostic of HH and can direct appropriate phlebotomy therapy.

3. The Screening of HH Family Members and Spouses.—Before direct HFE DNA analyses, family members of HH probands were typically evaluated by HLA serotyping to assess whether they inherited the same parental disease-associated haplotypes. In probands with C282Y homozygosity (~90% of patients with HH), the DNA test now allows the definitive categorization of at-risk family members as heterozygous, homozygous, or unaffected without the expense and complexity of HLA typing. This is particularly important for young family members who may not yet manifest phenotypic iron overload but for whom regular iron store monitoring may be essential for...
preventing serious complications. Direct DNA testing of the spouses of newly diagnosed homozygotes may even preclude the need to test the couple’s children if the spouse fails to carry a C282Y allele.39 Given both that most of those currently affected by HH are presently undiagnosed3 and that for every newly diagnosed HH case approximately 0.8 additional case will be discovered in family members,7 the proper use of the HH DNA test will likely help prevent a significant disease burden in young people.

4. Being Part of the Testing Algorithm for the Screening of High-Risk or Asymptomatic Populations for Iron Overload.—Hemochromatosis is perhaps the ideal disease for the implementation of a population-based screening programs because of the following reasons: (1) It is extremely common, affecting 0.3% to 0.8% of the population (∼1.5 million Americans). (2) It has high morbidity and mortality rates (if detected late). (3) Early phlebotomy therapy prevents chronic disease complications. (4) Therapy is effective, widely available, safe, and easy. (5) A sensitive screening test (TS) is inexpensive and widely available. (6) Early case detection has been shown to be ultimately cost-effective to society (by the prevention of chronic disease).40—43

The strong public health case for increased presymptomatic detection of HH cases has recently led the College of American Pathologists to recommend that all adults older than 20 years should now be screened for iron overload—initially by a TS test and, if TS levels are elevated, followed by confirmatory ferritin and/or liver biopsy evaluations.7 Since this recommendation was made just before the discovery of the HFE gene, the availability of the C282Y DNA test represents the major distinction between the official College of American Pathologists–endorsed algorithm7 and the updated screening algorithm in the Figure. Other physician and public health groups are evaluating similar screening endorsements. Until universal screening is more universally accepted, however, a more cautious approach (and one recently recommended by the Centers for Disease Control and Prevention and the National Institutes of Health44) should be to immediately begin TS-based hemochromatosis screening on all patients with potential symptoms of iron overload. If the initial TS measurement is elevated, appropriate follow-up could use SF, DNA, and/or liver biopsy tests in an algorithm similar to that shown in the Figure. These high-risk patients would include those with liver disease, arthritis, type 2 diabetes mellitus, cardiomyopathy, arrhythmia, weakness and fatigue, hypogonadism, or family histories of these disorders. The prevalence of HH in these high-risk groups will be greater than in asymptomatic persons, as will the positive predictive value of screening.44

Despite the disease prevention promise of universal HH screening, a number of critical questions remain unanswered. Foremost among these is our incomplete understanding of the risk, severity, and timing of disease expression in those with the homozygous genotype (ie, the clinical penetrance or positive predictive value). As an estimate, among C282Y homozygotes identified within families, 68% of females and 93% of males met strict clinical criteria for the expression of hemochromatosis (increased hepatic iron loads).38 Less stringent disease criteria before low-risk therapeutic phlebotomy (such as elevated SF levels) would define a higher penetrance of homozygotes with iron overload who might benefit from treatment. In contrast, nonfamilial cases would likely show a lower penetrance. The overall population clinical penetrance for C282Y homozygosity is then likely to be approximately 80%, but will clearly be dependent on the definition of disease and on nongenetic factors, including sex, age, and other concomitant medical or environmental risks (alcohol, hepatitis C, diet, occult blood loss, etc). Other critical hemochromatosis clinical research priorities include better determinations of the optimal HH screening algorithm (including the best TS cutoff and who, when, and where to screen), the costs (and benefits) of screening, the role of the DNA test in screening, and the ethical and social implications of the DNA test. In particular, as some of the C282Y homozygotes identified presymptomatically will be labeled as predisposed to HH but will never develop clinical disease, the real-world fears of insurance and employment discrimination and stigmatization must be addressed by proper legislative protections. The clinical and societal implications of defining millions of HH heterozygotes (∼12% of the population), some of whom will have iron overload,45 will clearly also need to be addressed. The soon-to-be initiated National Institutes of Health sponsored hemochromatosis genetic and phenotypic epidemiologic study of 100,000 adults (RFP NIH-NLHBI-HC-9905) will hope to answer some of these important questions.

In summary, the recent discovery of the HFE hemochromatosis gene and the high prevalence of its major disease-causing mutation (C282Y) have stimulated much additional interest in this historically underrecognized (but eminently treatable) chronic disease. Approximately 1.5 million Americans are currently affected with this disorder, most of whom are undiagnosed and continuing to accumulate toxic iron loads. The goals for diagnosing this disease have shifted during the past few years from the detection of clinically symptomatic patients (often with severe iron overload poorly amenable to therapy) to the detection of presymptomatic patients (with iron loads not yet at organ-damaging levels) who will respond more favorably to phlebotomy. Although the laboratory demonstration of phenotypic iron overload in the blood (TS and SF) and/or liver represents the key to recognizing mid- to late-stage disease, the most specific of these parameters (SF and liver biopsy) may not be elevated to diagnostic levels in the early (presymptomatic) stages of disease. The direct detection of the causative homzygous C282Y mutation (present in ∼90% of patients with HH) will then be of considerable clinical utility in both the diagnosis of patients in whom iron stores are already elevated and, perhaps more importantly, the detection of the millions of presymptomatic homozygotes for whom therapy may prevent future chronic disease.

References