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## Current Directions in Hemochromatosis Research: Towards an Understanding of the Role of Iron Overload and the *HFE* Gene Mutations in the Development of Clinical Disease

Since the discovery of a candidate gene (*HFE*) thought to be involved in the development of hereditary hemochromatosis, there has been much interest in the potential use of genetic testing as a screening tool for the disease in the general population. However, a recent study suggests that less than 1% of subjects who are homozygous for the gene mutations will go on to develop the full-blown disease of hereditary hemochromatosis, historically termed “bronzed diabetes.” The study also suggests that homozygotes have no higher risk of mortality or of any clinically significant morbidity than normal control subjects. This conclusion contradicts earlier findings that linked iron overload and *HFE* mutations to a number of devastating diseases, including cardiovascular disease, diabetes, and cancer.

**Key Words:** *HFE*, iron overload, hemochromatosis, gene mutations

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Hereditary hemochromatosis, a disorder affecting iron metabolism, was once believed to be a rare inheritable

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disease most often found in middle-aged or older men. Traditionally, patients were diagnosed with the disorder after presenting with signs and symptoms of severe iron overload, including hyperpigmentation of the skin, fatigue, arthralgias, cardiomyopathy, hepatic fibrosis, cirrhosis, and endocrinopathies, such as diabetes and gonadal failure (Table 1). It is now known that “bronzed diabetes” represents an advanced stage of disease and that the metabolic disorder is much more common than was originally thought. Hemochromatosis is, in fact, considered to be the most common genetic disorder in Caucasians of northern European origin.

Under normal physiologic conditions, the absorption of iron from dietary sources is highly influenced by the body’s requirement for the mineral. If body iron stores are depleted, dietary iron is more readily absorbed from the gastrointestinal tract. If, on the other hand, iron stores are plentiful, enterocytes absorb iron less efficiently, thereby helping to avoid iron overload and its toxic effects. In hereditary hemochromatosis, however, iron continues to be absorbed by the enterocytes at a high rate despite the presence of abundant iron stores in the liver and other tissues. In early stages of iron overload, patients are usually asymptomatic. With further accumulation of iron, patients may begin to experience symptoms such as fatigue, abdominal pain, and arthralgias, but these are likely to be nonspecific and insidious in onset. In advanced cases, iron overload can be a life-threatening condition, resulting in arrhythmias, liver failure, hepatomas, and insulin-dependent diabetes. If the disease is

**Table 1.** Clinical Manifestations of Hemochromatosis

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Depression
Fatigue
Weakness
Weight loss
Hypopituitarism
Hypothyroidism
Hyperpigmentation
Arrhythmias, heart disease
Diabetes mellitus
Adrenal insufficiency
Liver disease and hepatomas
Abdominal pain
Hypogonadism: amenorrhea, testicular atrophy, impotence, sparse body hair
Arthralgias

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identified before end-organ damage has been done, however, therapeutic phlebotomy can dramatically reduce the incidence of complications, and patients can expect to enjoy a normal lifespan.

Until recently, many patients with hereditary hemochromatosis went undiagnosed until the increasing iron burden had already produced irreversible tissue damage. In the last decade, however, advances in genetics and molecular biology have added to our armamentarium of diagnostic tools and have the potential to revolutionize the study, diagnosis, and treatment of hemochromatosis. These advances include the identification of a candidate gene, termed *HFE*, which is thought to play a critical role in iron metabolism. The *HFE* gene encodes for a protein that helps regulate iron uptake by enterocytes, but the mechanism by which this occurs is not fully understood. Several mutant forms of the gene have been identified and are thought to be associated with the development of hemochromatosis. In most studies, more than 92% of individuals diagnosed clinically with hereditary hemochromatosis have at least one of these mutations.<sup>1</sup> The first, called C282Y, involves a single nucleotide change in the human leukocyte antigen region on the short arm of chromosome 6, resulting in the substitution of tyrosine for cysteine at position 282 of the encoded protein. Burke et al. reviewed nine studies involving a total of 934 individuals with the clinical diagnosis of hereditary hemochromatosis, and 60 to 100% of subjects were found to be homozygous for the C282Y mutation.<sup>1</sup> Up to 14.9% of the remaining patients carried one C282Y mutation. A second *HFE* gene mutation, which results in a substitution of aspartic acid for histidine at position 63 (H63D), has been found in a much smaller percentage of subjects with hemochromatosis. Nevertheless, the presence of the mutation H63D is implicated in the development of clinical disease in a significant number of peo-

ple. In the review by Burke et al., up to 17.5% of subjects had at least one H63D mutation. Other *HFE* mutations have been reported in smaller numbers of patients, but their role in the development of disease has not yet been elucidated.

In the wake of the discovery of the *HFE* mutations, there has been much excitement about the potential uses of the genetic tests, which are now readily available. In many centers, genetic testing is routinely ordered for patients with physical exam findings, symptoms, or laboratory results that suggest an iron overload syndrome. Screening for the *HFE* genetic mutations has been found to be cost-effective in asymptomatic first-degree relatives of patients diagnosed with hereditary hemochromatosis.<sup>2</sup> Some clinicians have proposed an even more prominent role for genetic testing, suggesting that extensive population-based screening for the *HFE* mutant genes should be undertaken. Some have even suggested that tests for the *HFE* mutations be added to the routine screening panel for neonates.

However, a number of important points must be considered during the assessment of any disorder as a potential target for widespread screening programs. First, the disorder for which subjects will be screened should ideally be a relatively common one. Second, the disorder should cause a significant amount of morbidity or mortality when left untreated. Third, there must be an effective and acceptable treatment that can alter the course of the disease if it is diagnosed by a screening program. Advocates of population-based screening for hereditary hemochromatosis maintain that the disease and the proposed screening tests fulfill these criteria. First, hereditary hemochromatosis is thought to be the most common genetic disorder in whites of northern European extraction. If serum measures of iron status are used to determine the approximate prevalence of the disorder in these populations, the disease appears to occur in 2 to 5 out of every 1000 individuals. Second, patients who develop severe iron overload as a result of hereditary hemochromatosis have significant morbidity and mortality. Third, the treatment of iron overload by serial phlebotomy sessions is thought to be very effective in reducing morbidity and mortality. Ethical concerns have not allowed for randomized studies comparing phlebotomy with no treatment, but there is good evidence from retrospective cohort studies that patients who do not undergo the recommended phlebotomy have a shortened lifespan and increased morbidity when compared to those who have received treatment.<sup>3</sup> Lastly, therapeutic phlebotomy is relatively inexpensive, safe, and generally well-tolerated by patients. Hereditary hemochromatosis does indeed seem to be an excellent target for population-based screening.

Screening programs can only be successful if there

is a safe, readily available, cost-effective, and accurate screening tool designed to detect the disease. We now have noninvasive, widely available, and relatively inexpensive tests for the *HFE* mutations, and they have exciting potential as diagnostic tools for hereditary hemochromatosis. Nevertheless, there must be a better understanding of the role that the *HFE* mutations play in the development of clinical disease before widespread screening programs are instituted. In most studies, more than 92% of all subjects with the clinical diagnosis of hereditary hemochromatosis carried at least one of the genetic mutations. Up to 8.5% of subjects did not possess an *HFE* mutation, however, and they would therefore have escaped detection during a genetic screening program. In addition, the penetrance of the gene mutations is uncertain. In the past, it was often assumed that homozygosity for the C282Y mutation would inevitably produce the clinical syndrome of hemochromatosis, in the absence of chronic blood loss or therapeutic phlebotomy. However, scientific evidence for this assumption was scant.

In 1999, the subject of *HFE* gene penetrance was addressed in a large population-based study by Olynyk et al.<sup>4</sup> In this study, 3011 free-living people in Western Australia were screened for the presence of the genetic mutation C282Y and for evidence of iron overload with measurements of serum transferrin saturation and ferritin. Persons with at least one C282Y mutation or abnormal iron studies were also tested for the presence of the H63D mutation. Sixteen (0.5%) of the 3011 subjects were found to be homozygous for the C282Y mutation, 359 (11.9%) were heterozygous for the C282Y mutation, and 65 (2.2%) were compound heterozygotes, with one C282Y and one H63D mutation. All 16 homozygotes had either an elevated ferritin level (>300, present in 12 out of 16 patients) or abnormal transferrin saturation (>45%, present in 15 out of 16 patients). Eleven of these patients then underwent liver biopsy, and all were shown to have an elevated hepatic iron index. Three of the 11 patients undergoing biopsy were found to have hepatic fibrosis and one had cirrhosis. Half of the homozygotes were reported to have clinical characteristics of the disorder, including hyperpigmentation and arthritis. This study therefore supported the long-held belief that C282Y homozygotes will routinely develop iron overload, as defined by laboratory measures of iron status. On the other hand, only 50% of the homozygotes had overt signs or symptoms of iron overload, and they were not compared with a control group without the disease. The clinical significance of the metabolic derangement in this group was thus unclear. These patients might have developed symptoms over time if they were not treated for iron overload, or perhaps the disease might never have

manifested itself clinically. Further study is clearly indicated.

A recent study by Beutler et al. was also designed to investigate the penetrance of the hemochromatosis genes.<sup>5</sup> This large study involved 41,038 members of the Kaiser Permanente medical plan in San Diego, CA, each of whom had presented for a routine outpatient exam. Each person completed a written questionnaire designed to detect common symptoms of hemochromatosis, including fatigue, joint symptoms, skin pigmentation, liver problems, impotence, and diabetes. A comprehensive physical exam was then performed and all subjects were tested for the C282Y and H63D gene mutations, iron stores, and glucose. All C282Y homozygotes and a number of control subjects also underwent testing for blood levels of AST and type IV collagen, a marker of hepatic fibrosis. Serum type IV collagen has been shown to be a reliable indicator of hepatic fibrosis and cirrhosis in patients with hemochromatosis.<sup>6</sup> This marker was used in the study by Beutler et al. in lieu of the more invasive gold standard, the liver biopsy.

Of the 41,038 individuals screened, 152 (0.4%) were found to be C282Y homozygotes and 616 (1.5%) were identified as compound heterozygotes. Of the homozygotes in this study, 75% of the men and 40% of the women had transferrin saturation greater than 50%. Ferritin was greater than 250  $\mu\text{g/L}$  in 76% of the homozygous men and greater than 200  $\mu\text{g/L}$  in 54% of the homozygous women. Eight percent of homozygotes had an AST greater than 40 and 25.8% had an elevated serum type IV collagen level, suggestive of the presence of hepatic fibrosis. On the other hand, most classic symptoms of hemochromatosis did not occur more frequently in homozygotes or compound heterozygotes than in normal controls, despite the laboratory evidence of iron overload. For example, fatigue was reported by 27.4% of all homozygotes, 26.4% of compound heterozygotes, and 26.5% of normal controls. Hyperpigmentation of the skin was noted by 1.6% of homozygotes and 5.1% of compound heterozygotes versus 7.6% of controls. Complaints of joint problems occurred in 36.3% of homozygotes, 42.9% of compound heterozygotes, and 41.6% of controls. Likewise, diabetes, abdominal pain, impotence, depression, weight loss, hair loss, and arrhythmias were not found to be more common in homozygotes or compound heterozygotes than in normal individuals. Even in subgroups of patients over age 55 and patients with laboratory evidence of iron overload (i.e., high ferritin level or transferrin saturation), these symptoms were not more common than in controls. Only a history of liver problems was reported slightly more frequently by homozygotes than by control subjects. By the authors' estimation, less than 1% of homozygotes develop the classic phenotype of hemochromatosis. This is in striking

contrast to current teaching about the disease and, if true, has important implications for the proposed population-based screening programs. If the penetrance of the gene were indeed so low, widespread screening programs based on genotype would ultimately benefit a much smaller number of individuals than originally thought.

The current study was large and well designed, but there are a few weaknesses that should be addressed. First, the paper's title, "Penetrance of 845G → A (C282Y) *HFE* hereditary hemochromatosis mutation in the USA," is somewhat misleading because the studied population does not fully reflect the diversity of the American people. The study involved subjects who were enrolled in a San Diego HMO, either through employers or through Medicare. Seventy-seven percent of the study group was Caucasian, but non-Hispanic whites compose only 71% of the American population today.<sup>7</sup> Only 3.7% of the study group was black, but blacks account for 12.8% of the U.S. population. Hispanics were only slightly underrepresented in the study population. Because hereditary hemochromatosis is more common in Caucasians, the racial makeup of the study group might lead to an overestimation of prevalence of the *HFE* mutant genes in the U.S. population. Furthermore, racial differences may affect gene penetrance and iron absorption. Therefore, it seems unwise to rely solely on these results to generate screening recommendations for the entire population of the United States. Additional studies investigating gene penetrance in other segments of the population could be helpful in either supporting or refuting the conclusions reached by Beutler et al.

Second, 45 of the homozygotes identified in this study had been previously diagnosed with hereditary hemochromatosis during a screening program that used ferritin levels and transferrin saturation to identify patients with iron overload. They had then undergone therapeutic phlebotomy treatments prior to their participation in the study by Beutler et al. In the study, pretreatment labs, exams, and questionnaires were used whenever they were available. However, pretreatment questionnaires were not available for 28 of these individuals (62% of the patients previously diagnosed or 18% of all homozygotes in this study), so they were not included in the analysis of data. Could this exclusion of a large number of homozygotes with iron overload have influenced the results of the study? The analysis did include newly diagnosed patients with elevated iron stores, who did not report signs or symptoms of the disorder more frequently than normal controls. Therefore, it can probably be assumed that the 28 previously diagnosed individuals would have been asymptomatic prior to diagnosis, but the study would have been stronger if pretreatment surveys had been available for those patients.

Finally, in asserting that only 1% of homozygotes will be clinically affected by the disorder, the authors suggest that the biochemical evidence of iron overload found in up to 76% of male homozygotes and 54% of female homozygotes is inconsequential. They also dismiss the finding that nearly 26% of homozygotes had laboratory evidence of hepatic fibrosis, noting that there was no apparent decrease in survival amongst homozygotes in this study, even at advanced ages. They concluded, "the liver abnormalities detected had little or no effect on survival." Indeed, these patients may not have been symptomatic at the time of this study, and they might never develop the full syndrome of "bronzed diabetes," but they do have evidence of abnormal iron metabolism. It has been suggested that elevated body iron stores or serum iron levels may play a role in the development of a variety of diseases, including cardiovascular disease<sup>8</sup> and cancer.<sup>9</sup> Other studies have found an association between heterozygosity for C282Y and an increased risk of cardiovascular disease,<sup>10</sup> stroke,<sup>11</sup> diabetes,<sup>12</sup> diabetic nephropathy,<sup>13</sup> and all-cause mortality.<sup>14</sup> A number of other studies, on the other hand, have found no such association between elevated iron stores or possession of a C282Y mutation and disease. For example, one 1997 study concluded that higher iron stores are actually associated with a lower risk of cardiovascular disease and all-cause mortality.<sup>16</sup> The recent study by Beutler et al. did not find an increased prevalence of diabetes in subjects with at least one *HFE* mutation or in those with iron overload, and it also failed to find a survival disadvantage in either homozygotes or heterozygotes. Unfortunately, risk for cardiovascular disease, stroke, cancer, and diabetic nephropathy was not directly addressed in this study. In light of the conflicting results seen in other studies, it seems too early to presume, as the authors of the current study have done, that possession of an *HFE* mutation and the resulting elevated iron stores are of no consequence to overall health. Hemochromatosis patients with laboratory evidence of iron overload would presumably benefit from education, careful monitoring, and perhaps even therapeutic phlebotomy, at least until the relationship between iron stores, *HFE* genotype, and disease is more fully explored and understood.

Despite these weaknesses, the recent study by Beutler et al. is clearly an important one. It raises critical questions about the relationship between phenotype and genotype, and as a result, it has significant implications for hemochromatosis screening programs. Clearly, there is not enough evidence to promote *HFE* genetic screening programs in the general population at this time, although there is an indication for testing in select patients, such as those with signs or symptoms suggestive of hemochromatosis, evidence of abnormal iron metab-

olism, liver disease, or a family history of hemochromatosis. Nevertheless, it seems too early to conclude that the possession of an *HFE* mutant gene has no significant adverse effect on morbidity or mortality. Additional studies are needed to further our understanding of hereditary hemochromatosis and to determine whether the *HFE* mutations play a significant role in the development of clinical disease.

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