

Hereditary Hemochromatosis Since Discovery of the *HFE* Gene

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Background: Hereditary hemochromatosis is an inherited disorder of iron metabolism that is characterized by excessive iron deposition in major organs of the body. Chronic increased iron absorption leads to multiorgan dysfunction. Since the discovery of the gene responsible for the majority of cases, research has progressed rapidly to identify the gene product, the effects of mutations, and the implications for different populations. The protein product of the *HFE* gene is a transmembrane glycoprotein, termed HFE, that modulates iron uptake. Mutations in the *HFE* protein compromise its function and produce disease symptoms. Two mutations, C282Y and H63D, have been linked to the majority of disease cases.

Approach: We reviewed the recent literature for the molecular basis of hereditary hemochromatosis. Genotypic information was combined with biochemical and clinical phenotypic information to achieve a better understanding of the disease mechanism.

Content: This review provides a comprehensive discussion of known mutations in the *HFE* gene and their phenotypic expression. Diagnostic criteria using molecular genetic techniques in conjunction with traditional biochemical tests are provided. Current methods and limitations of molecular testing are examined in detail. A strategy for population screening and an algorithm for diagnosis that incorporates molecular testing are presented. Treatment by therapeutic phlebotomy and the use of blood obtained from hemochromatosis patients are discussed.

Summary: Although the disease mechanism has not been completely elucidated, phenotypic and penetrance data are becoming available. Controversy still exists concerning the role of genetic testing in diagnosis and population screening.

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Hereditary hemochromatosis, an inherited disorder of iron metabolism, is one of the most common genetic diseases in individuals of Northern European descent, affecting 1 in every 200–300 individuals. It is an autosomal recessive disorder that is expressed more severely in males than in females. Individuals with hemochromatosis chronically absorb excess iron. The result is iron overload and potential injury to involved organs. Clinical symptoms vary among affected individuals, ranging from mild, if any, symptoms to life-threatening heart and liver disease. The genetic defect, therefore, demonstrates reduced penetrance in the population and variable clinical expression. The discovery of a candidate gene responsible for hemochromatosis (the *HFE* gene) in 1996 stimulated research and has increased understanding of this disorder. This review describes current knowledge of the molecular pathology, biochemistry, and genetic basis of the disease. Criteria for screening and diagnosis of hereditary hemochromatosis are discussed.

Clinical Significance

Symptoms of hemochromatosis are the result of iron accumulation in a variety of organs. Iron accumulation leads to injury and organ failure. Although hereditary hemochromatosis varies in clinical severity, the most common presenting feature is fatigue. Other clinical features of iron accumulation include arthritis, cardiac arrhythmias or heart failure, diabetes mellitus, hepatic cirrhosis, hyperpigmentation, hypothyroidism, hypogonadism, and less commonly, hepatocellular carcinoma (1). Symptoms often begin after the age of 50, although early symptoms are very heterogeneous and may be difficult to detect. Atypical cases are frequent. Marked iron accumulation typically occurs before specific symptoms and signs are recognized. Young asymptomatic patients have significantly lower tissue iron, but iron concentrations are abnormally high for their ages. Clinical heterogeneity exists even among members of the same family. The rate of iron accumulation and the extent of iron overload vary within families (2).

Symptoms occur more frequently in males than females, with an estimated male-to-female ratio of 3:1 (3).

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Received October 17, 2000; accepted April 23, 2001.

The decreased incidence of symptoms in females is partially attributable to the protective effects of menstrual blood loss and pregnancy in premenopausal women. Females may also be underdiagnosed because they may present with different symptoms than males. Although females can exhibit the complete phenotype, they usually present with fatigue and pigmentation, whereas men usually present with cirrhosis and diabetes (3).

***HFE* Mutations**

An association between hemochromatosis and HLA-A3 and genetic linkage to the HLA complex was first suggested in 1976 (4). Twenty years later, the *HFE* (HLA-H) gene was identified as a candidate gene for hereditary hemochromatosis (5). The most common mutation described is a G-to-A transition at nucleotide 845 (G845A), which substitutes a tyrosine for a cysteine at amino acid position 282 (designated C282Y). The allele frequency of C282Y in the Caucasian population is 0.063 (6). The association of C282Y homozygosity with hereditary hemochromatosis is dependent on the population. In studies of individuals of European descent, C282Y homozygosity in hemochromatosis patients ranged from 64% in Italian patients (7) to 100% in Australian patients (8). C282Y homozygosity occurs in 80–90% of English and French hemochromatosis patients (5, 9, 10).

The C282Y mutation is associated with a specific haplotype in nearly all European populations, consistent with a mutation occurring over 2000 years ago (11). The C282Y mutation is either absent or has low allele frequencies in non-Caucasian populations, i.e., African, Asian, South Pacific, and Aboriginal Australian populations (12, 13). Most C282Y alleles in these populations share the same haplotype with Northern European populations (13). This suggests that these mutations arose in non-Caucasian populations because of genetic admixture. C282Y mutations described in some Australian patients (8) and in Sri Lanka (11) are associated with haplotypes other than the ancestral haplotype in northern Europeans. The fact that this mutation arose independently suggests selective pressure, possibly allowing a heterozygous advantage against anemia or an infectious agent.

Some hemochromatosis patients have a C-to-G transversion at nucleotide position 187 (C187G). This mutation, known as H63D, substitutes an aspartic acid for histidine and has an allele frequency of ~16% in the European population (5, 9, 10). Although this mutation appears to have little effect when inherited alone, it may contribute to the disease when inherited with the C282Y mutation, producing a compound heterozygous genotype. In compound heterozygotes, the two mutations are inherited on separate chromosomes (in trans). To date, only one incidence of H63D and C282Y inherited on the same chromosome (in cis) has been reported (14). However, many compound heterozygotes in the general population are asymptomatic (8, 9). In hemochromatosis patients who are compound heterozygotes, a second unidentified mu-

tation in the *HFE* gene or an as yet unknown gene may be responsible for symptoms. However, the incidence of compound heterozygotes in hemochromatosis patients is higher than expected if H63D or a genetically linked modifier did not contribute to the phenotype (5, 15). The C282Y/H63D compound heterozygous genotype is postulated to have a reduced penetrance [estimated to be 0.44–1.5% (5, 9, 10)]. Combined allele and genotype frequencies for C282Y and H63D are listed in Table 1. Although none of the random controls in the initial studies were homozygous for C282Y, larger studies have indicated that the frequency of C282Y homozygotes in the general population is <0.5% (16, 17).

A third base alteration that replaces serine with cysteine (S65C) is present in ~1.5% of the European population (6, 18). Although S65C is considered a benign polymorphism, a C282Y/S65C genotype may confer a slight increase in disease risk, contributing to a mild disease phenotype (19). Additional studies are necessary to confirm the role of this genotype and its possible penetrance.

Other rare *HFE* mutations have been reported in individual families. Two mutations, G168T and G169A, were found in an Italian population. When these mutations were inherited in trans with C282Y, the compound heterozygotes had signs of hemochromatosis (20). Both of these mutations produce a truncated *HFE* protein and may explain the reduced percentage of C282Y homozygosity in hemochromatosis patients in the Italian population. Other isolated mutations have been found in C282Y heterozygotes with classic phenotypes. Two families in Alabama have missense mutations (G93R and I105T) (21). In one European family, a splice-site mutation causes skipping of exon 3 (22). Another mutation, T281K, has been described in cis with a C282Y mutation (23). In this case, this patient's symptoms were most likely attributable to C282Y homozygosity, and therefore, the effect of this mutation remains unknown. The population frequen-

Table 1. Allele and genotype frequencies.

Amino acid changes		Allele frequency, %	
High-penetrance allele	Low-penetrance allele	Hemochromatosis patients ^a	Random controls ^a
Cys282Tyr (C282Y)	His63Asp (H63D)	85 (C282Y) 4.9 (H63D)	5.0 (C282Y) 15 (H63D)
Genotype		Genotype frequency, %	
WT/WT ^b	WT/WT	7.3	64
C282Y/C282Y	WT/WT	82	0.0 (0.5) ^c
WT/C282Y	WT/WT	2.0	8.6
WT/WT	H63D/H63D	1.4	3.1
WT/WT	WT/H63D	2.5	23
WT/C282Y	WT/H63D	4.5	1.3

^a Average calculated from published US (10), French (9), and Italian (7) hemochromatosis populations and random controls.

^b Wild-type allele.

^c From population-based screening studies (16, 17).

cies and effects of these rare mutations have yet to be determined.

Phenotypic Expression of *HFE* Mutations

Discovery of the *HFE* gene permitted investigation of the effects of mutations on the disease phenotype. For C282Y homozygosity, reports range from 75% to 96% penetrance for iron overload; however, these studies differ in their definition of iron overload, with most using increased transferrin saturation (TS)¹ and biochemical or histologic hepatic iron index values (24–26). Increased serum ferritin (SF) and characteristic histologic features of the liver also have been used (24, 26). These studies estimated that penetrance for other symptoms is ~50%. In these studies, the majority of asymptomatic C282Y homozygotes were premenopausal females. Of the C282Y homozygotes biopsied [those with increased transferrin (Tf) or ferritin], increased hepatic iron was seen in all samples. Because penetrance is age related and gender influenced, a genotype/phenotype correlation and phenotypic expression are more accurately addressed by age and gender. When stratified by these factors, penetrance of C282Y homozygosity in men >40 years of age is 95% for iron overload. Fifty percent of these men have other symptoms. In men younger than 40 years, 80% have iron overload and 12% have additional symptoms. Iron overload is seen in 80% of C282Y homozygous women older than age 40. Approximately 13% of these women have other symptoms. Fewer women (39%) younger than age 40 have iron overload; in this study, these women had no other symptoms (27). The overall prevalence of clinically significant iron overload in patients who were not homozygous for C282Y or did not have other explicable causes is reported as 0.13% (26).

Phenotypic expression of H63D is even more complex. Fewer than 2% of C282Y/H63D compound heterozygous individuals show clinical symptoms, and those symptoms are mild and mainly present in males. Increasing serum iron concentrations and TS are seen in wild-type, H63D heterozygotes, C282Y heterozygotes, H63D homozygotes, C282Y/H63D compound heterozygotes, and C282Y homozygotes, in that order. However, only C282Y homozygotes have a significant increase (26, 28). Although cirrhosis occurs, hepatic cancer is rarely seen in compound heterozygotes. It has been suggested that the H63D mutation may be a predisposing factor for iron accumulation only in combination with other genetic (such as C282Y heterozygosity) and/or environmental conditions (28).

Non-*HFE* Hemochromatosis

Genetic heterogeneity of hemochromatosis is shown by multiple mutations within the *HFE* gene as well as by other genes, particularly in specific populations. In some

populations, hemochromatosis is not associated with the C282Y mutation. In particular, Chinese hemochromatosis patients do not have the C282Y mutation (29), although it is not known whether other mutations in the *HFE* gene or mutations in a different gene are responsible. Because of interactions with *HFE*, β_2 -microglobulin (β_2m) and the Tf receptor (TfR) are candidates for additional hemochromatosis genes; however, mutations have not been demonstrated in either gene (30, 31).

In studies of iron overload in sub-Saharan Africans or African Americans, neither of these populations showed linkage to the *HFE* gene (32, 33). These patients also show slightly lower serum iron concentrations and TS than seen in Caucasian hemochromatosis patients.

Family studies of hemochromatosis patients who do not have *HFE* mutations can be used to identify other hemochromatosis genes. One such gene has been found in a Sicilian population (34). The gene *TFR2* (*HFE3*) maps to chromosome 7q22. The causative mutation is a C750G nonsense mutation that encodes a truncated protein (Y250X). This gene is poorly understood in iron metabolism, but it has 66% homology with the TfR (*TFR*) gene. The *TFR2* protein may be involved in iron uptake rather than iron regulation, as is postulated for the *HFE* protein.

Another hemochromatosis gene is suspected in an Italian family and may help explain the reduced percentage of C282Y homozygotes in the Italian hemochromatosis population. Affected family members have abnormal TS or SF and increased iron concentrations. This pedigree shows no linkage to *HFE* (35), indicating that another gene is responsible for hemochromatosis in this family.

Although hereditary, juvenile hemochromatosis is considered a separate disease from hereditary hemochromatosis. In juvenile hemochromatosis, clinical symptoms manifest before age 30, both sexes are affected equally, and endocrine failure and cardiomyopathy occur. The juvenile form maps to chromosome 1q (36).

HFE Protein

The protein product of the *HFE* gene is a 343-residue type I transmembrane glycoprotein that resembles the MHC class I proteins in sequence and three-dimensional structure (37–39). Both the *HFE* and MHC class I proteins contain a membrane-bound heavy chain with three extracellular domains ($\alpha 1$, $\alpha 2$, and $\alpha 3$). The $\alpha 1$ and $\alpha 2$ globular domains form a platform composed of an eight-stranded antiparallel β -sheet topped by two α helices. This platform is positioned on top of an immunoglobulin constant-like $\alpha 3$ domain that binds β_2m noncovalently. In the MHC proteins, the $\alpha 1$ and $\alpha 2$ helices form a groove in which peptides can bind. Unlike the MHC proteins, *HFE* does not bind peptides. Crystallographic data show that the *HFE* $\alpha 1$ helix lies closer to the $\alpha 2$ helix, forming a groove that is shallower and narrower than the MHC peptide-binding groove (37). This physical difference reflects the different protein function for *HFE* in cellular Tf-mediated iron uptake (40).

¹ Nonstandard abbreviations: TS, transferrin saturation; SF, serum ferritin; Tf, transferrin; β_2m , β_2 -microglobulin; TfR, transferrin receptor; and Tf-Fe, diferric transferrin.

A cluster of four histidine residues at the base of the $\alpha 1$ domain on the protein surface resembles the composition of iron-binding sites in several proteins, but no such function has been elucidated for these residues (37). Instead, HFE is thought to regulate iron uptake by a mechanism that involves pH-dependent binding to the TfR protein (40). According to this mechanism, cells acquire iron in the form of diferric Tf (Tf-Fe). TfR, the homodimeric membrane receptor (41), binds an iron-rich Tf molecule at the cell surface pH of 7.4. The TfR:Tf-Fe complex enters the cell by receptor-mediated endocytosis. At the acidic pH of endosomes (6.2), iron is released from Tf and is transported to the cytoplasm. The Tf and TfR proteins cycle back to the cell surface (41).

Although the exact molecular mechanism is contested, HFE is thought to bind to TfR and influence Tf-Fe-mediated intracellular iron delivery (40). One model proposes that the wild-type HFE protein decreases the affinity of TfR for iron-bound Tf (40). Initial studies using both soluble and membrane-bound forms of the protein show that the association of HFE with TfR reduces the apparent binding affinity of TfR for Tf-Fe by 5- to 10-fold [Fig. 1A; Ref. (40)]. However, the concentration of Tf is sufficiently high that the receptor is completely saturated under physiologic conditions, suggesting that this may be an unlikely mechanism. Other researchers have found that HFE blocks the binding of Tf to TfR and is responsible for negative regulation of iron uptake (42, 43). In this model, HFE associates with TfR at or near the Tf-binding site and competitively inhibits the TfR:Tf-Fe interaction. Binding involves interaction of an HFE $\alpha 1$ domain helix and adjacent loop with helices 1 and 3 of the helical domain of TfR. The expression of HFE reduces the number of Tf-binding TfR molecules on the cell surface, i.e., HFE decreases the functional receptor number rather than ligand affinity [Fig. 1B; Ref. (42)]. In a third model, it is proposed that HFE limits iron uptake by preventing complete release of iron from the HFE:TfR:Tf-Fe complex in the endosome. In proteins with C282Y mutations [Fig. 1C; Ref. (44)].

The effects of the HFE mutations C282Y and H63D on protein structure and function have also been studied.² The C282Y mutation converts a cysteine residue in the $\alpha 3$ domain to a tyrosine residue (5). This mutation prevents the formation of a disulfide bond and alters folding of the HFE protein. The mutant HFE protein does not bind $\beta 2m$. Failure to interact with $\beta 2m$ impairs protein processing, transport, and cell surface expression (45). The mutant protein is retained in the endoplasmic reticulum and mid-Golgi compartments. It fails to undergo late Golgi

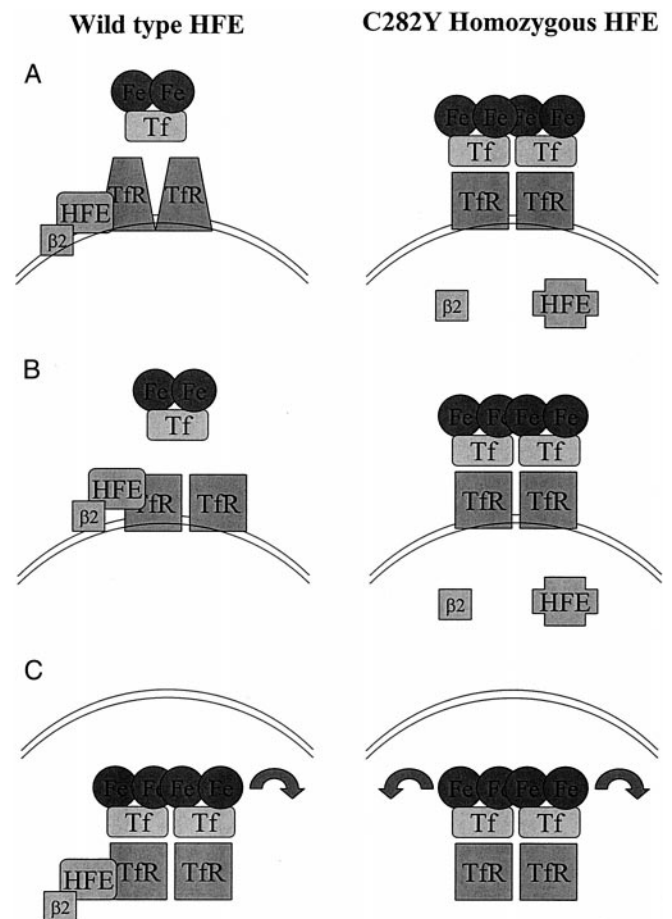


Fig. 1. Binding of HFE protein to TfR.

(A), wild-type HFE protein binds to TfR and decreases the binding affinity of TfR for iron (Fe)-rich Tf. The C282Y mutation prevents association of HFE and $\beta 2m$ ($\beta 2$). HFE is not transported to the cell surface and does not affect TfR:Tf-Fe binding. (B), wild-type HFE protein binds to TfR at or near the Tf-binding site and competitively inhibits the TfR:Tf-Fe interaction. In proteins with C282Y mutations, HFE is not transported to the cell surface and does not affect TfR:Tf-Fe binding. (C), wild-type HFE binds to TfR and limits iron uptake by preventing complete release of iron from the HFE:TfR:Tf-Fe complex in the endosome. In proteins with C282Y mutations, HFE does not affect iron release from the TfR:Tf-Fe complex.

processing and is subject to accelerated degradation (45). The result is loss of protein function.

The H63D mutation converts a histidine residue to aspartate in an $\alpha 1$ domain loop. Amino acid substitution at this site prevents formation of a His-Asp salt bridge and disrupts local protein structure (45). This mutant protein is expressed at the cell surface, but does not have the same interaction with TfR that the wild-type protein does. As a result, more iron is deposited in cells that usually rely on HFE for modulation of iron intake. This is evidence that the H63D mutations affects protein function (40).

Diagnostic Criteria

Hereditary hemochromatosis must be distinguished from other conditions that cause iron overload, such as porphyria cutanea tarda, β -thalassemia, medicinal and transfusional iron overload, and African iron overload (46).

² In protein crystallography studies, numbering of the protein sequence begins at the first residue of the mature protein rather than at the first residue of the hydrophobic signal sequence of the primary translation product. The mutations referred to as C282Y and H63D are residues Cys-260 and His-41 in the mature protein. To maintain consistency with the genetic nomenclature, we use the nomenclature from the primary translation product.

Traditionally, TS, SF, and hepatic iron concentrations have been used to diagnosis this condition clinically. Measurement of serum iron and total iron binding capacity on a fasting, morning specimen should be performed as the initial test (1). TS is expressed as the ratio of serum iron concentration and total iron binding capacity. A SF assay should be performed to evaluate total body iron content. In the absence of other potential causes of iron overload, persistently increased TS (TS $\geq 50\%$ for premenopausal females and TS $\geq 60\%$ for males or postmenopausal females) and ferritin (SF $> 200 \mu\text{g/L}$ for premenopausal females or SF $> 300 \mu\text{g/L}$ for males and postmenopausal females) are suggestive of hereditary hemochromatosis (47). A serum TS of 55% has a sensitivity of 90% in detecting homozygous C282Y males (25), although most authors recommend TS $> 60\%$ as a threshold. Although similar in sensitivity to TS, a SF concentration is less specific (25, 26) because increased SF is seen in other disorders as well as hemochromatosis.

In the past, iron overload was confirmed by the detection of increased hepatic iron in a liver biopsy specimen (47). Liver biopsy specimens are assessed for iron overload by histochemical or biochemical means. Histochemical staining is easier and far less expensive, but determination of the iron content per gram of dry weight of liver tissue is more precise. Results of either method are adjusted for the age of the affected individual to produce an index value. A histochemical index threshold of 0.15 and a biochemical index threshold of 2.0 may be used to distinguish hemochromatosis from other causes of iron overload, such as alcoholism (46), in patients with normal liver histology and function. These indices are not accurate for patients with compromised liver function (cirrhosis). Although a liver biopsy may be used to assess prognosis in some clinical cases and to detect increased hepatic iron in subclinical cases, a biopsy is an invasive procedure and may not be desirable, particularly in subclinical cases for an otherwise healthy individual. Liver biopsies may be useful for patients with additional risks factors for liver disease (48).

With the availability of genetic testing, hemochromatosis may be confirmed by genotyping. Genotyping is considered $> 99\%$ sensitive for a given mutation. Specificity is more difficult to quantify in genetic testing because of incomplete phenotype/genotype correlation. However, because phenotypic and clinical features of hereditary hemochromatosis show wide variations, genotype could be evaluated with clinical features to better define hemochromatosis. Combining the molecular defects with clinical features for disease classification has been very effective for other genetic diseases (49). A combined genotype/phenotype approach would promote study of atypical hemochromatosis patients to identify modifying environmental and/or genetic factors that contribute to or protect from disease. For detection of C282Y homozygosity, a TS of 50% has a sensitivity of 0.52 and a specificity of 0.908. Increased ferritin ($200 \mu\text{g/L}$ in women and 250

$\mu\text{g/L}$ in men) has a sensitivity of 0.70 and a specificity of 0.803 (6). Family members of individuals diagnosed with hemochromatosis should be screened to detect subclinical cases. Presymptomatic detection is important because early treatment can prevent organ damage. However, preclinical disease is more difficult to determine by TS and ferritin because reference intervals for these analytes are dependent on age, gender, and diet.

Molecular Testing

Hemochromatosis mutation detection can supplement TS and SF concentrations to confirm the diagnosis in symptomatic patients and to detect subclinical cases. Although genotyping is more expensive than measuring Tf or ferritin concentrations, false-positive or -negative results because of diet, alcoholism, or other conditions are greatly reduced. Molecular analysis detects individuals at risk for developing hemochromatosis independent of environmental factors. Mutation detection, however, does not predict severity of disease or age of onset. Pedigree analysis is important, especially in cases of hemochromatosis in patients who do not have the C282Y/C282Y or the C282Y/H63D genotype. These families may have other mutations in the *HFE* gene or mutations in other genes that contribute to the clinical phenotype. In these rare cases, family members should be screened using TS or SF concentrations.

Most molecular diagnostic laboratories test for C282Y and H63D mutations, although some laboratories test for H63D only in C282Y heterozygous cases to detect compound heterozygotes. A variety of molecular methods have been reported for detecting *HFE* mutations. Most methods are based on amplification of the specific gene region by PCR, followed by mutation detection. The most common method uses a restriction enzyme to differentiate alleles. For C282Y and H63D, the mutations create a restriction site; therefore, the enzyme cuts the allele containing the mutation but not the wild-type allele. Gel electrophoresis separates the mutant from the wild-type sequence based on nucleic acid fragment size. Allele-specific amplification is another commonly used method. In this method, PCR primers specific for either the wild-type or mutant allele are designed so that the mutation position is close to the 3' end of the primer. Each primer will amplify only its specific allele. The presence or absence of amplification by the specific primers identifies the genotype. A third method uses hybridization probes labeled radioactively or fluorescently. The probes are complementary to either the wild-type or mutant sequence and hybridize to the PCR product. Differing hybridization conditions and temperatures allow differentiation of the mutant and wild-type alleles. An example of C282Y and H63D results using hybridization probes multiplexed in a single reaction is seen in Fig. 2.

Although false positives and false negatives are rare, they can occur in molecular testing. A polymorphism near the 3' end of one of the original primers described by

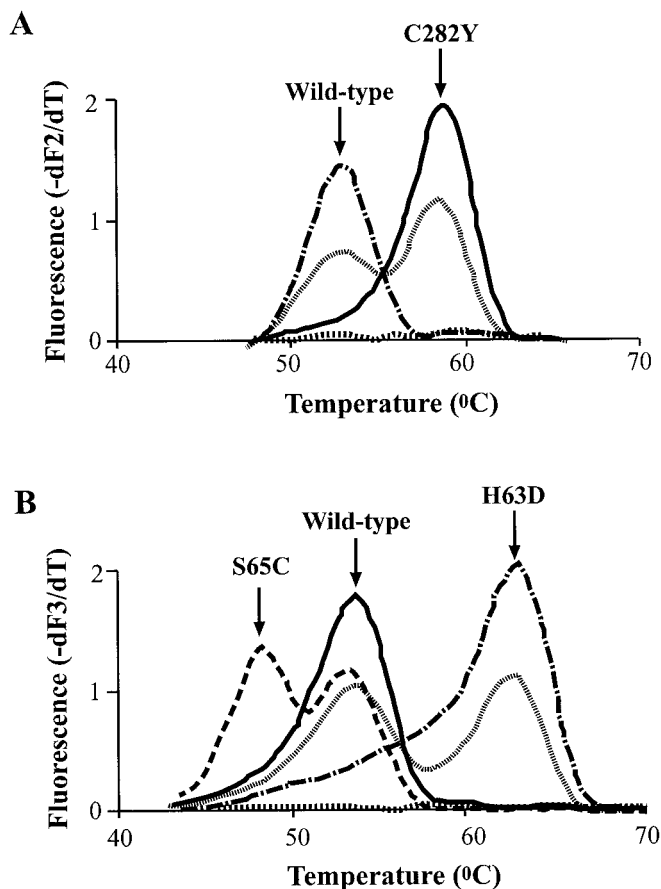


Fig. 2. Multiplexed C282Y and H63D genotyping using hybridization probes and melting peak analysis.

C282Y and H63D were amplified using rapid-cycle PCR. After amplification, PCR products were denatured, cooled to 45 °C, and then slowly heated with continual fluorescence monitoring. The hybridization probes dissociated at characteristic melting temperatures for the wild-type and mutant alleles. Melting curve data were converted to derivative curves or "melting peaks". (A), C282Y genotype: ■■■, no-template control; ■■■■, wild type; ■■■■, homozygous C282Y; |||, heterozygous C282Y. (B), H63D genotype with S65C distinguished from the wild-type and H63D: ■■■, no-template control; ■■■, wild-type; ■■■■, homozygous H63D; |||, heterozygous H63D; ■■■■, heterozygous S65C.

Feder prevents the primer from annealing to the DNA template and extending during PCR (50). This hinders amplification of the wild-type allele. The result is an allele-specific amplification of the C282Y allele in heterozygotes, giving a false-positive homozygous rather than a heterozygous genotype for C282Y. Since the discovery of this polymorphism, one concern is that C282Y homozygosity is overestimated because of allele-specific amplification. However, several laboratories have reported no allele-specific amplification with these primers, probably because of less stringent PCR conditions that allow a mismatched primer to anneal and extend (51). Using a primer that does not include this polymorphism eliminates the possibility of a false positive attributable to this polymorphism. Mutations within restriction sites, primers, or probe sequences are an inherent source of diagnostic error in molecular testing, although some methods can distinguish between mutations (18, 23).

Population Screening

Because of the high frequency of these mutations in the population, the American Medical Association recommends establishing guidelines for population screening. Hemochromatosis meets the criteria for population screening in that it is medically significant, a reliable diagnostic test is available, and an accepted treatment can prevent serious complications. However, population screening is not universally accepted because the natural history and burden of suffering of the disease is unknown (47).

Population screening by molecular methods is not advised because of uncertainties of phenotypes and genetic privacy and psychological issues (47). Some of these issues are currently being addressed. New phenotypic data show a high penetrance of iron overload for C282Y homozygous males (85%) and females (69%) in relatives of hemochromatosis patients (52). Privacy of genetic information remains a concern because of the potential for insurance and job discrimination. Many individuals cite insurance concerns for refusing genetic testing. Although anecdotal evidence of genetic discrimination exists, no studies are available to determine the extent to which such discrimination occurs. A concern about psychological stresses attributable to genetic testing is also being studied. In studies of subjects at risk for Huntington disease and inherited breast cancer, which, as hemochromatosis, are adult-onset diseases but which carry a greater potential of burden of suffering, subjects who knew their genotypes, whether mutation carrier or mutation negative, showed either no increase or a decrease in stress when compared with subjects who did not know their genotypes (53, 54).

For the general population, cost-effective screening can be accomplished by measuring TS followed by a DNA test (55). A TS $\geq 60\%$ ($\geq 50\%$ for premenopausal females) or a serum iron concentration $>27 \mu\text{mol/L}$ ($>150 \mu\text{g/dL}$) is an indication for additional testing. Oral or parenteral iron supplementation should be discontinued and testing repeated. Increased TS on two occasions is suggestive of hemochromatosis (46). Unbound iron-binding capacity to preselect individuals for genotyping has been proposed as a cost-effective screening strategy (16). In this study, initial screening by unbound iron-binding capacity detected more C282Y homozygotes, produced fewer false positives, and cost less than the use of TS. For individuals with clinical or phenotypic features of hemochromatosis, a positive DNA test (C282Y homozygosity or C282Y/H63D compound heterozygosity) is diagnostic. However, a negative DNA test does not completely rule out hemochromatosis attributable to other mutations in *HFE* and other genes. A biopsy may be needed in cases of repeatedly increased TS or SF to confirm iron accumulation. Family members of an affected individual should be screened by DNA testing to detect subclinical cases once *HFE* mutations are confirmed in the proband (family member who first presented with the disease).

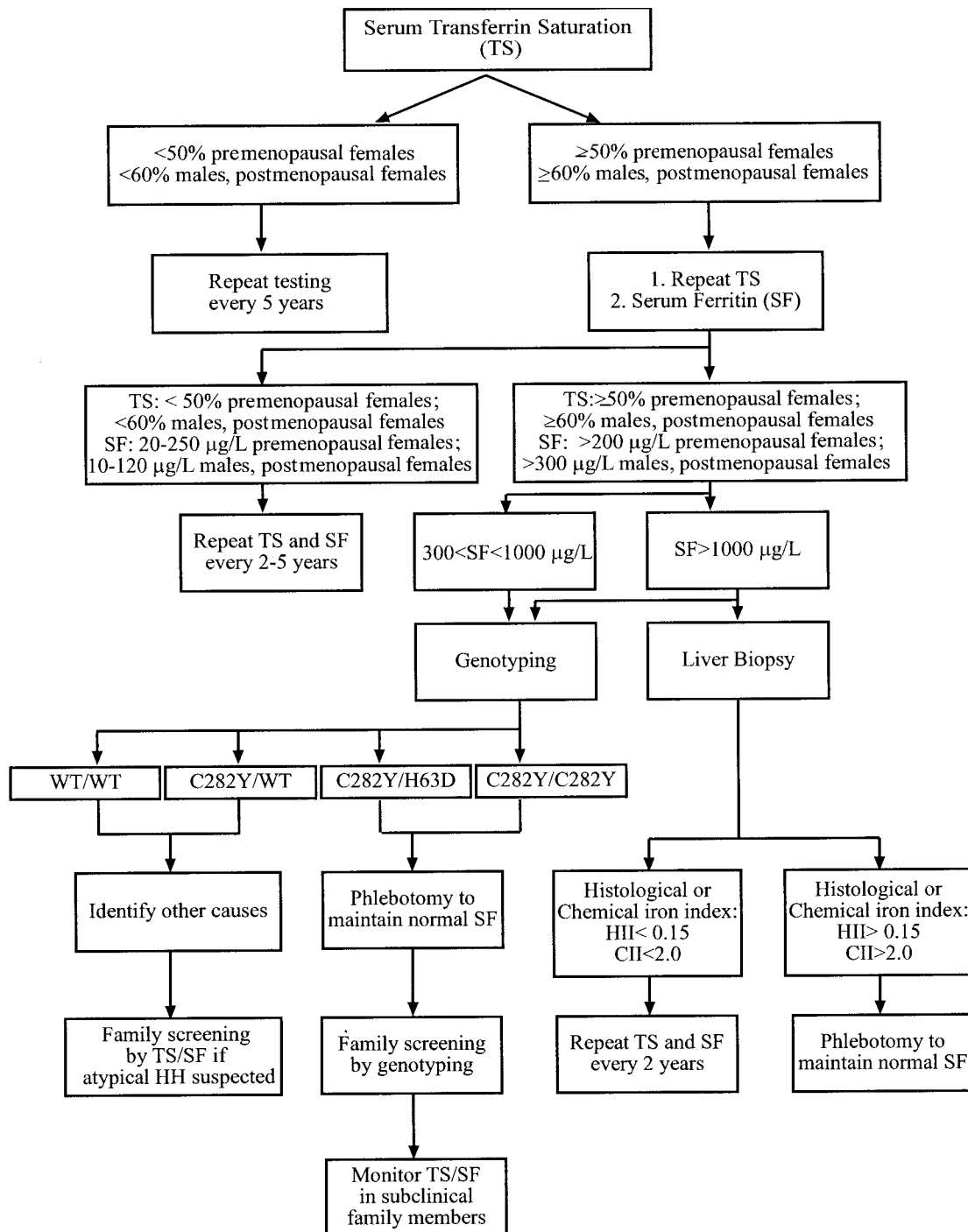


Fig. 3. Algorithm for screening and diagnosis of hemochromatosis.

WT, wild type; HII, histologic iron index; CII, chemical iron index; HH, hereditary hemochromatosis.

Because genetic testing is available, liver biopsies are not required for a diagnosis of hemochromatosis. Liver biopsies are indicated for C282Y homozygotes with suspected liver disease, C282Y homozygotes or heterozygotes with high SF ($>1000 \mu\text{g/L}$), patients negative for C282Y mutations with unexplained iron overload, and patients with additional risk factors for liver disease (48).

Both serum TS and SF concentrations are relatively insensitive to iron accumulation, especially in younger individuals and women. In addition, they may be falsely increased as a result of diet, alcohol consumption, or other forms of liver disease. Therefore, screening and diagnosis should not be based on a single measurement. An algorithm for the diagnosis of hereditary hemochromatosis

that incorporates *HFE* mutation analysis is depicted in Fig. 3. This approach begins with serum TS. If the value is increased, this test should be repeated on a fasting sample in conjunction with a SF measurement. If the TS is repeatedly increased and the SF is significantly increased (more than twice the upper limit of the reference interval), further testing is recommended. Liver biopsy, phlebotomy, or genetic testing can be used to establish a diagnosis. Traditionally, individuals with increased TS and SF values have been evaluated by liver biopsy or trial of phlebotomy (56). Since the discovery of the *HFE* gene, direct DNA genotyping has become available. The depicted algorithm emphasizes genotyping, although there is no consensus for a recommended algorithm. If the DNA test confirms the presence of a mutation, family members should be tested. If no mutation is detected, a liver biopsy may be performed in individuals with consistently increased TS and SF concentrations to detect atypical hemochromatosis. For those patients with SF concentrations within the reference interval, TS and SF concentrations should be repeated at 2- to 5-year intervals, proceeding through the algorithm if both are subsequently increased.

Treatment

Benefits of early treatment include increased survival and prevention of complications (46). Treatment for both symptomatic and presymptomatic patients consists of therapeutic phlebotomy. Initial treatment for symptomatic patients consists of weekly or biweekly phlebotomies of up to one unit (450 mL) of blood, if tolerated. Iron depletion is marked by early iron deficiency anemia with microcytosis and decreased serum TS and SF concentrations. SF concentrations usually normalize before TS does. Iron depletion may require up to 1 to 2 years, depending on the initial iron load. Maintenance therapy consists of phlebotomy at intervals of 2 to 6 months, depending on SF concentrations.

Use of blood obtained from hemochromatosis patients for transfusion purposes is controversial. During depletion therapy, hemoglobin and hematocrit values are often lower than the values recommended by the American Association of Blood Banks. However, units of blood collected during maintenance therapy may be suitable (57). Establishments that want to use blood from hemochromatosis therapy need to obtain an exemption from the Food and Drug Administration. This variance must include verification that the patient was not billed for the procedure as well as safety data regarding viral marker rates, incidence of transmissible infections based on rates of seroconversion to viral markers, frequency of postdonation reports of undisclosed risks, and adverse events for both donor and recipient (58).

In conclusion, the discovery of the *HFE* gene has allowed a better understanding of hereditary hemochromatosis. The causative mutation has been confirmed in studies worldwide. The role of the low-penetrance mutation

H63D is being elucidated. The effects of mutations on iron stores and clinical symptoms are unfolding for precise penetrance data. Direct DNA genotyping is available to aid diagnosis. In addition, other mutations and other genes are being implicated in hemochromatosis. Protein structure and function studies have followed the discovery of the gene. The mutational effects on the protein are revealing the pathology of hemochromatosis. Research since the discovery of the *HFE* gene has increased our knowledge of this common, potentially life-threatening, but treatable inherited disease.

We greatly appreciate the valuable comments of Ronald L. Weiss, MD, MBA; and Carl T. Wittwer, MD, PhD.

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