

REVIEW ARTICLE

MEDICAL PROGRESS

Hereditary Hemochromatosis — A New Look
at an Old Disease

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FOR MUCH OF THE 20TH CENTURY, HEREDITARY HEMOCHROMATOSIS WAS regarded as a clinically and genetically unique entity. The classic findings on presentation — diabetes, bronze pigmentation of the skin, and cirrhosis — were first described in the 19th century, when the term “hemochromatosis” was first used¹⁻³; by 1935 it had become clear that the disease was hereditary and was caused by excess deposits of iron in the tissue.⁴ In the 1970s and 1980s, it was recognized as an autosomal recessive disorder linked to the region of the short arm of chromosome 6 encoding HLA-A*3,^{5,6} and in 1996 “the hemochromatosis gene,” *HFE*, was finally identified.⁷

In the few years since the discovery of the *HFE* gene, our understanding of hereditary hemochromatosis and of human iron metabolism in general has become much more complete (Fig. 1). We know that mutations in other genes that control iron metabolism can cause similar forms of iron overload (defined in terms of excess body iron levels) that lead to deposits of iron in the tissues with distinct patterns and organ-damaging potential. Genetic testing has revolutionized the diagnosis of hereditary hemochromatosis and has revealed that the phenotypic expression of a given mutation in an iron-metabolism gene may vary widely. These advances have stretched the boundaries of the historical definition of hereditary hemochromatosis. The Online Mendelian Inheritance in Man (OMIM) database,⁸ for example, currently lists four types of hereditary hemochromatosis, each caused by mutations involving a different gene (Table 1). Is the label “hereditary hemochromatosis” valid in all cases? Can these disorders be adequately classified into types exclusively on the basis of their underlying mutations, without consideration of their unifying clinical features?

In this article I review some of the important features of classic *HFE*-related hereditary hemochromatosis (still the most common form in most populations of European descent) — features that are shared by other phenotypic forms of hereditary hemochromatosis, forms that are classified in the OMIM database as types 2A, 2B, and 3. These features are indeed absent from other known forms of hereditary iron overload, including the form currently listed in the OMIM database as hereditary hemochromatosis type 4.

PHENOTYPIC AND GENETIC FEATURES

CLASSIC HEREDITARY HEMOCHROMATOSIS

Classic hereditary hemochromatosis is an autosomal recessive iron-overload disorder associated with mutation of the *HFE* gene, which is located on chromosome 6; in most cases the mutation is a single-base change that results in the substitution of tyrosine for cysteine at position 282 of the *HFE* protein (C282Y).⁷ C282Y seems to have originated by chance in a single Celtic (or Viking) ancestor in northwestern Europe some 2000 years ago. The genetic defect, which caused no serious obstacle to reproduction and may

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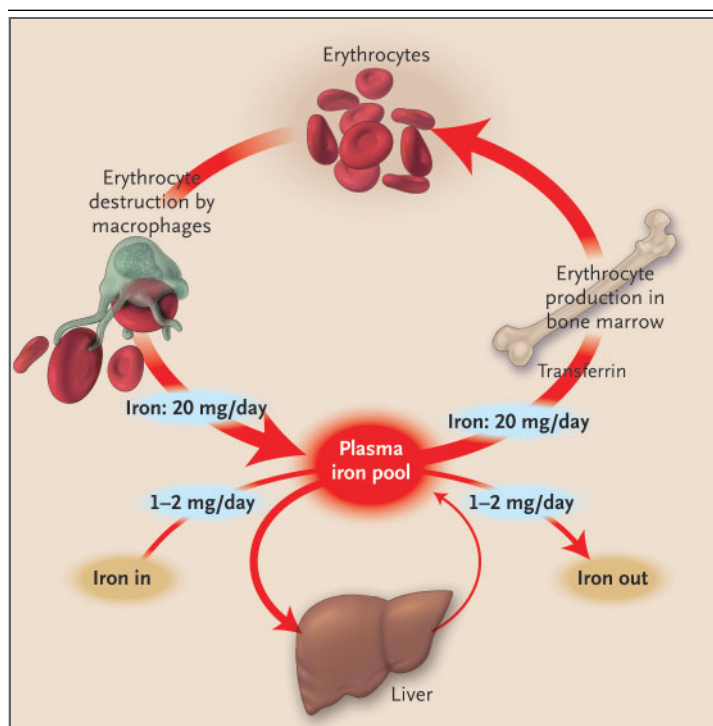


Figure 1. Normal Iron Homeostasis in Humans.

Plasma levels of iron are closely regulated to ensure a daily supply of approximately 20 mg to the bone marrow for incorporation into hemoglobin in erythroid precursors and mature red cells. Most of the iron found in the plasma derives from the continuous breakdown of hemoglobin in senescent red cells by reticuloendothelial macrophages. Approximately 1 to 2 mg per day is also taken up by duodenal enterocytes and transferred to the plasma compartment or, depending on body needs, stored in the enterocytes as ferritin. These stores are eliminated when enterocytes are sloughed at the end of their life cycles; apart from menstrual blood loss, this is the only significant means by which excess body iron is excreted. Iron recycled by macrophages (as well as that absorbed from the gut) is loaded onto serum transferrin and delivered primarily to the bone marrow for reincorporation into new red-cell precursors. The remaining body iron (approximately 1000 mg) is stored, primarily in hepatocytes.

even have conferred some advantages (e.g., resistance to dietary iron deficiency and certain infectious diseases), was passed on and spread by population migration. Homozygosity for the C282Y mutation is now found in approximately 5 of every 1000 persons of northern European descent — a prevalence 10 times that of cystic fibrosis genotypes.^{9,10}

All persons who are homozygous for the C282Y mutation are genetically predisposed to a chain of events that may culminate in severe damage to multiple organs, but it is currently impossible to predict whether, and to what extent, the mutation will be phenotypically expressed. The natural history of

classic hereditary hemochromatosis involves a gradual, highly variable, stepwise progression that depends on numerous individual variables (Fig. 2). In a small percentage of C282Y homozygotes, laboratory evidence of altered iron metabolism never develops.

Symptomatic organ involvement (when it occurs) generally begins in midlife, often with nonspecific symptoms such as unexplained fatigue or joint pain. Liver disease (ranging from slightly elevated aminotransferase levels, with or without hepatomegaly, to cirrhosis and even hepatocellular carcinoma) usually predominates, but endocrine disorders (diabetes, hypogonadotropic hypogonadism, impotence, and hypothyroidism), cardiac problems (arrhythmias and heart failure), and joint disease (destructive arthritis) are also found. Although iron metabolism is abnormal, erythropoiesis is not jeopardized, and hematologic anomalies are not usually seen. Therapeutic phlebotomy is usually effective in reducing stores of both plasma iron and tissue iron, and even aggressive phlebotomy generally poses no risk of anemia to the patient.

Other mutations in *HFE*, less common than C282Y, have also been described. The clinical effects of a mutation in which aspartic acid replaces histidine at position 63 (H63D), for example, appear to be limited,¹¹ although 1 to 2 percent of persons with compound heterozygosity for C282Y and H63D seem predisposed to disease expression.¹⁰ The clinical significance of other, rarer forms of compound heterozygosity, such as heterozygosity for C282Y and a mutation in which cysteine replaces serine at position 65 (S65C) or heterozygosity for H63D and S65C, is still controversial.¹²⁻¹⁴

GENETIC AND PHENOTYPIC VARIATIONS

Although relatively few cases have been described to date,¹⁵⁻¹⁸ the iron-overload phenotype associated with mutations in the gene encoding transferrin receptor 2 (*TfR2*) appears to be very similar to that of classic, *HFE*-related hemochromatosis. These two forms of the disease are representative of adult-onset hereditary hemochromatosis, which is characterized by gradual iron loading, a relatively late onset of parenchymal iron deposition, and predominantly hepatic organ damage.¹⁹

The juvenile-onset phenotype is much more severe. Plasma iron loading and tissue iron excesses (reflected by increased transferrin-saturation values and serum ferritin levels, respectively) are evident early in life in both sexes. Functional iron-metabo-

Table 1. Comparative Overview of the Primary Iron-Overload Disorders Classified as Hereditary Hemochromatosis in the OMIM Database.*

Feature	HFE-Related Hereditary Hemochromatosis†		Juvenile Hereditary Hemochromatosis		TfR2-Related Hereditary Hemochromatosis		Ferroportin-Related Iron Overload‡
	Type 1	Type 2, subtype A	Type 2, subtype B	Type 3	Type 4		
OMIM classification	HFE, 6p21.3	HJV (originally called HFE2), 1q21	HAMP, 19q13.1	TfR2, 7q22	SLC40A1, 2q32		
Implicated gene and its chromosomal location							
Gene product	HFE	Hemojuvelin	Hepcidin	Transferrin receptor 2	Ferroportin (also iron-regulatory protein, or metal-transporter protein)		
Known or postulated function§	Interaction with transferrin receptor 1, probably facilitating uptake of transferrin-bound iron; possibly modulation of hepcidin expression	Unknown; possibly modulation of hepcidin expression	Down-regulation of iron release by enterocytes, macrophages, or placental cells	Possibly uptake of iron by hepatocytes	Export of iron from enterocytes, macrophages, placental cells, or hepatocytes		
Pattern of inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal dominant		
Evidence of expanded plasma iron compartment (high transferrin saturation)	Earliest detectable biochemical anomaly	Earliest detectable biochemical anomaly	Earliest detectable biochemical anomaly	Earliest detectable biochemical anomaly	Only in advanced stages		
Main organs accumulating iron	Liver, endocrine glands, heart	Liver, endocrine glands, heart	Liver, endocrine glands, heart	Liver, endocrine glands, heart	Liver, spleen		
Predominant cell distribution of iron accumulation	Parenchymal	Parenchymal	Parenchymal	Parenchymal	Reticuloendothelial		
Potential for organ damage	Variable	High	High	Variable	Low		
Anemia	No	No	No	No	May be seen in menstruating women or after phlebotomy		
Response to therapeutic phlebotomy	Excellent: decrease in serum ferritin in parallel with transferrin saturation; no risk of anemia	Excellent: decrease in serum ferritin in parallel with transferrin saturation; no risk of anemia	Excellent: decrease in serum ferritin in parallel with transferrin saturation; no risk of anemia	Excellent: decrease in serum ferritin in parallel with transferrin saturation; no risk of anemia	Fair: rapid decrease in transferrin saturation with persistently high serum ferritin; substantial risk of anemia with aggressive phlebotomy regimen		
Decade of onset of symptomatic organ disease	4th or 5th	2nd or 3rd	2nd or 3rd	4th or 5th	4th or 5th		

* OMIM denotes Online Mendelian Inheritance in Man.

† Other common names are hereditary hemochromatosis, classic hemochromatosis, and HLA-linked hemochromatosis.

‡ Other common names are autosomal dominant hemochromatosis, ferroportin disease, and autosomal dominant reticuloendothelial iron overload.

§ There may be other, as yet unknown, functions related to iron overload. The listed functions do not, at least at this time, always account for the known pathophysiological features associated with the gene mutation.

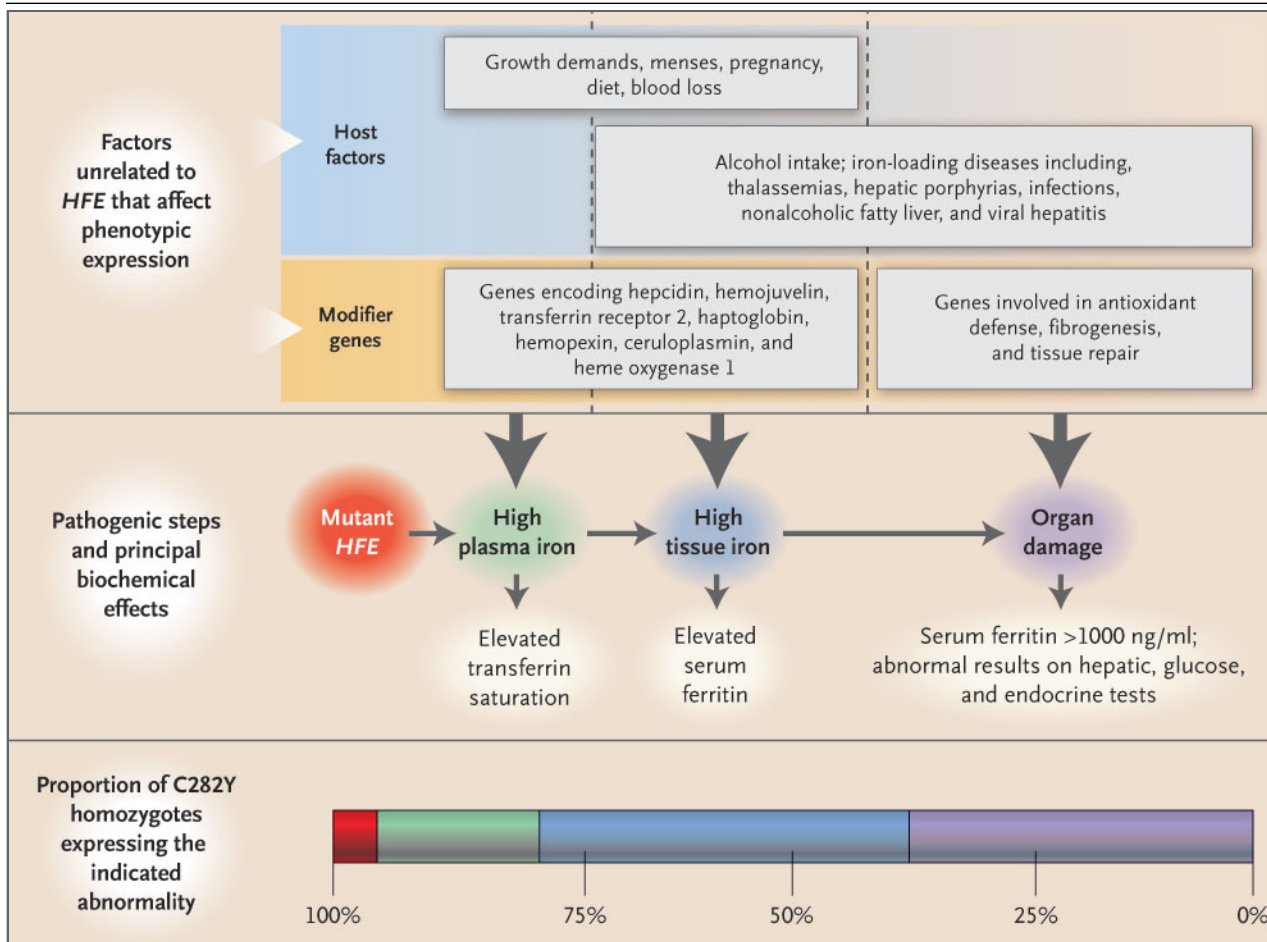


Figure 2. HFE-Related Hereditary Hemochromatosis, a Multistep, Multifactorial Iron-Overload Disorder.

Most persons homozygous for the C282Y mutation have progressive expansion of the plasma iron compartment, as reflected by increasing saturation of transferrin, the plasma iron transporter (middle panel). (Transferrin saturation is calculated as a percentage as follows: $100 \times \text{serum iron level (in micrograms per deciliter)} \div \text{total iron-binding capacity (in micrograms per deciliter)}$.) HFE-related hereditary hemochromatosis develops slowly and is rarely evident before adulthood. As shown in the top panel, its progression may be accelerated by high dietary iron intake or attenuated by active iron utilization or iron loss. The “protective effects” of menstruation and pregnancy probably explain why plasma iron loading in girls and young women is more gradual than it is in older women and why the rate of symptomatic organ disease in boys and men is three times the rate in women of reproductive age.

In some cases, disease expression never goes beyond this biochemical phase, but in more than half of all C282Y homozygotes, the plasma iron overload is followed by a second phase, one that involves progressive accumulation of iron in the parenchymal tissues of the liver and other organs, as heralded by rising serum levels of ferritin. The iron-deposition rate is also influenced by various factors, including the circulating level of hepcidin, the iron-regulatory hormone, and the activity of proteins involved in the metabolism of heme (e.g., hemopexin and haptoglobin) and non-heme iron. The third phase, which occurs in a limited percentage of cases (bottom panel) and is more common in men than in women, is manifested by markedly elevated levels of serum ferritin along with signs and symptoms of target-organ impairment. This outcome is related to the toxic effects of redox-active iron but is greatly facilitated by other, concomitant cytotoxic insults and by hypersusceptibility to oxidant damage and fibrogenesis.

lism data from patients with juvenile-onset disease are limited, but estimated rates of iron accumulation markedly exceed those observed in the adult-onset forms.²⁰ Liver biopsies and autopsies reveal a parenchymal iron distribution resembling that seen in both HFE- and Tfr2-related disease.²¹ Symptomatic organ involvement occurs as early as the second decade of life. Although liver involvement is a constant feature, diabetes, hypogonadotropic hypogonadism, cardiomyopathy, arrhythmias, and heart

failure are far more evident than in the adult-onset form.^{22,23} This difference may reflect different susceptibilities to massive iron overload among the developing organs. Death, usually from intractable heart failure, before the age of 30 years is not uncommon.

Rare cases of juvenile hereditary hemochromatosis have recently been linked to a homozygous mutation in the *HAMP* gene,²⁴ which encodes hepcidin, a peptide that plays a key role in human iron metabolism. However, most juvenile-onset cases have been mapped to chromosome 1q,²⁵ where the gene that produces hemojuvelin, *HJV* (originally called *HFE2*), has recently been identified.²⁶ Deleterious *HJV* mutations have been documented in families with 1q-linked juvenile hemochromatosis. The phenotypic similarities between *HJV*- and *HAMP*-related disease are reflected in their OMIM classification as hereditary hemochromatosis subtypes 2A and 2B, respectively (whereas the adult forms caused by *HFE* and *TfR2* mutations are listed separately as types 1 and 3 — one of the inconsistencies of this classification).

THE GENETIC CAST: MAJOR PLAYERS AND SUPPORTING ACTORS

Human iron homeostasis depends on the coordinated functions of numerous genes, the precise roles of which are, in many cases, still obscure. *HFE* is a good example. The major-histocompatibility-complex class I–like protein, HFE, which has an ancestral peptide-binding groove that is too narrow for antigen presentation,²⁷ is also incapable of binding iron.²⁸ Newly synthesized HFE binds to beta₂-microglobulin,²⁹ an event necessary for its expression on the cell surface and endosomal membranes, where it interacts with transferrin receptor 1 (TfR1), the major receptor for transferrin.^{28,30} By disrupting a disulfide bond in HFE that is critical for its binding to beta₂-microglobulin,²⁹ the C282Y mutation impairs cell-surface expression of HFE and the interaction of HFE with TfR1. In vitro findings reported in the late 1990s indicated that normal HFE diminished the ligand-binding affinity of TfR1 and that HFE competed with transferrin for iron uptake.^{31,32} However, more recent studies of cultured macrophages from patients with hereditary hemochromatosis, macrophage cell lines,^{33,34} and cells overexpressing both HFE and beta₂-microglobulin³⁵ suggest that HFE normally facilitates, rather than hinders, TfR1-mediated cellular uptake of transferrin-bound iron. It now seems that HFE

might also bind to other, uncharacterized proteins or exert direct effects on endosomal iron transport.^{36,37}

Even less is known about the function of transferrin receptor 2 (TfR2). Cloned in 1999,³⁷ TfR2 shows 66 percent homology with TfR1, and in transfected cells, it mediates the uptake of transferrin-bound iron,³⁸ possibly through a mechanism similar to that described for TfR1. TfR2 differs from TfR1 in its affinity in vitro for transferrin (1/25 to 1/30 as strong),³⁹ its high level of expression on hepatocytes, and the fact that its expression is not down-regulated by hepatic iron overload.^{40,41}

The severity of the phenotype in juvenile hereditary hemochromatosis suggests that there are underlying genetic alterations involving proteins that play more critical roles in iron homeostasis than either HFE or TfR2, and hepcidin certainly fits this description. Hepcidin is synthesized by hepatocytes in response to both inflammatory stimuli and iron overload.⁴²⁻⁴⁴ Studies in transgenic mice indicate that it has a key role in down-regulating the intestinal absorption and placental transport of iron and the release of iron by macrophages.⁴⁵ It has been hailed as “the iron-regulatory hormone,” although the mechanisms underlying its effects are unclear. Very little is known about the newly identified *HJV* gene, but current observations suggest that its product, hemojuvelin, modulates hepcidin expression.²⁶

PATHOPHYSIOLOGY

PATHOGENETIC MODELS OF HFE-RELATED HEREDITARY HEMOCHROMATOSIS

Increasing transferrin-saturation values, the earliest detectable biochemical abnormality in classic hereditary hemochromatosis,^{46,47} reflect a gradual but progressive expansion of the plasma iron compartment, a change traditionally attributed to increased intestinal absorption. Duodenal transfer of iron to the plasma is inappropriately high for body iron stores.⁴⁸ In normal persons, phlebotomy triggers sharp, transient increases in the rate of iron absorption (from 1 to 2 mg per day to 5 mg per day), which help to ensure iron supplies in the bone marrow. In hereditary hemochromatosis, this response is exaggerated (with the rate of iron absorption reaching 8 to 10 mg per day), and it remains high for years.⁴⁹ Erythropoietic needs are fully satisfied, but the enterocytes continue to transfer unneeded iron from digested matter into the bloodstream, instead of retaining it in the form of ferritin. This shift may

reflect a primary pathogenic abnormality in the enterocytes themselves or a disruption of regulatory signals originating at another site. In either case, it may be responsible for the relatively iron-deficient phenotype observed in these cells, which contain far less ferritin than other epithelial cells (e.g., hepatocytes) in a given patient's body.^{50,51} It also may explain why physiologic shedding of these enterocytes does little to reduce excess body iron (Fig. 1). The end result is intestinal absorption of iron that generally exceeds iron loss by approximately 3 mg per day.⁵²

The crypt-programming model was elaborated in 1997 after immunohistochemical studies showed normally high HFE expression in undifferentiated enterocyte precursors at the base of duodenal villi.⁵³ In this model, the relative iron deficiency of mature absorptive enterocytes and increased intestinal iron absorption are attributed to abnormal interaction between TfR1 and mutant HFE in crypt cells (Fig. 3A and 3B).

Findings suggestive of increased iron transport at the basolateral membrane of enterocytes in hemochromatosis has emerged from numerous studies of HFE-related hemochromatosis in humans⁵⁵⁻⁵⁸ and in mice.⁵⁹ Although some of these studies^{55,56} also revealed evidence of augmented luminal iron transport, it is possible that the predominant alteration in transport involves altered release of iron from the basolateral side, rather than abnormal luminal uptake. Furthermore, although the gut clearly contributes to the circulatory iron overload in hereditary hemochromatosis, macrophages are normally a much more important source of plasma iron than are enterocytes.⁶⁰ Macrophages in hereditary hemochromatosis are also relatively iron-poor, and their iron-metabolism gene-expression profiles are consistent with the presence of iron deficiency⁶¹; they release more iron⁶² and retain less transferrin-bound iron than their normal counterparts³³ — a defect that can be corrected by the introduction of normal HFE.³³ Although the crypt-programming theory makes no provision for this possibility, impaired functionality of HFE, resulting in inappropriate iron release, might conceivably be a defect shared by enterocytes and macrophages in hereditary hemochromatosis.

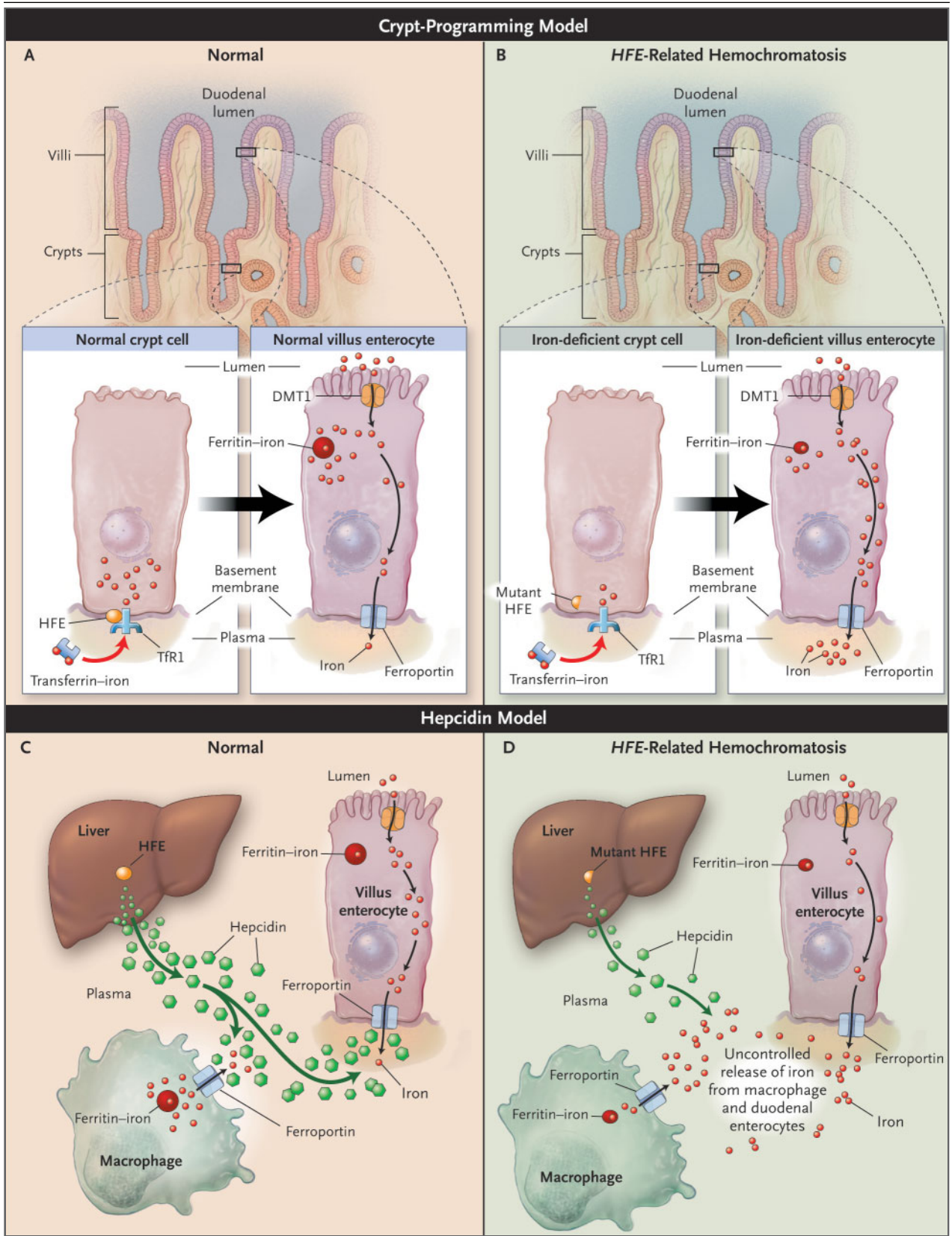
The crypt-programming hypothesis has been actively challenged since the discovery of hepcidin⁴²⁻⁴⁴ and its association with juvenile hereditary hemochromatosis.²⁴ Given the key role of hepcidin in the down-regulation of iron release by enterocytes and

Figure 3 (facing page). Pathogenic Models of HFE-Related Hereditary Hemochromatosis.

In the crypt-programming model, under normal conditions (Panel A), undifferentiated duodenal crypt cells, which are not exposed to dietary iron, take up iron from the bloodstream to support their growth, a process mediated by transferrin receptor 1 (TfR1), which is located on their basolateral membranes. (TfR1, which normally dimerizes, is shown for simplicity as a monomer.) In this model, iron uptake by crypt cells is modulated, in relation to the iron saturation of circulating transferrin, by the interaction of TfR1 with normal HFE. The cellular pool of iron accumulated in this manner "programs" the iron-absorbing capacity of the mature absorptive enterocytes that will be derived from these cells by modulating the activity of specialized luminal iron transporters (e.g., divalent metal transporter 1, [DMT1]) and basolateral iron transporters (e.g., ferroportin).⁵⁴ Normally programmed daughter cells absorb small percentages of dietary iron from the intestinal lumen, transfer to the plasma the amount needed to compensate for daily iron losses, and retain the rest as ferritin. According to this model, the presence of HFE with the C282Y mutation, which is unable to interact with TfR1, leads to iron-deficient crypt cells, which give rise to iron-deficient daughter cells (Panel B). These cells are programmed to react to iron starvation by hyperactively and persistently absorbing iron from the intestinal lumen and transferring virtually all of it into the bloodstream, regardless of actual erythropoietic needs.

In the hepcidin model (Panel C), the rate of iron influx into the plasma depends primarily on the activity of hepcidin: when plasma iron levels are high, the synthesis of hepcidin increases, diminishing the release of iron from enterocytes and macrophages, possibly through interaction with iron-export proteins, such as ferroportin. When plasma iron levels drop, the synthesis of hepcidin is down-regulated, allowing these cells to release increased amounts of iron. The stimulus and mechanisms underlying the modulation of hepcidin synthesis are currently unknown, but it is likely that HFE has a role. In the presence of a normal *HAMP* gene, mutant HFE might somehow alter the currently uncharacterized signals or factors for appropriate synthesis or up-regulation of hepcidin by hepatocytes, leading to uncontrolled release of iron from macrophages and duodenal enterocytes. As is typical of adult-onset hereditary hemochromatosis, the rate of plasma iron loading would be more gradual than that provoked by the complete absence of circulating hepcidin (a feature that is typical of the juvenile-onset phenotype).

macrophages,⁴⁵ the severe early-onset iron-loading phenotype associated with its absence is not surprising. However, recent data show that hepatic expression and serum levels of hepcidin are also inappropriately low in patients with adult-onset HFE-related disease.^{63,64} Furthermore, its expression in the liver is substantially impaired in HFE-



knockout mice,⁵⁹ and hepatic iron deposition can be prevented in this model by hepcidin overexpression.⁶⁵ These findings have led to the proposal of an alternative model, one in which hepcidin emerges as a central pathogenic factor in classic hemochromatosis (Fig. 3C and 3D).

This model might explain the hypothesis, mentioned earlier, that plasma iron loading in hereditary hemochromatosis stems from inappropriate release of iron not only by enterocytes (as emphasized in the crypt-programming model) but also by macrophages. Circumstantial evidence suggests that HFE may be required for hepcidin synthesis and regulation, but the details of this relation are obscure. Iron overload is clearly associated with evidence of increased hepcidin synthesis in mice⁴⁴ and humans,⁶⁶ but the mechanisms underlying this up-regulation are unknown. Hepatocytes appear to respond to iron *in vivo*,⁶⁴ but *in vitro* exposure of hepatocytes to ferric iron⁴⁴ or iron-saturated transferrin⁶⁶ does not increase the expression of hepcidin messenger RNA (mRNA) and may even reduce it. Thus, other cells may serve as iron sensors. The probable involvement of macrophage-derived interleukin-6 in the induction of hepcidin mRNA expression during infection⁶⁶ suggests that reticuloendothelial macrophages (e.g., Kupffer's cells) might be good candidates. Nevertheless, it is presently unclear whether induction of hepcidin during iron overload requires functional HFE in Kupffer's cells, in hepatocytes, or in both.

HEPCIDIN: THE KEY PLAYER IN ALL FORMS OF HEREDITARY HEMOCHROMATOSIS?

The involvement of hepcidin has been documented (if not fully characterized) in two variants of hereditary hemochromatosis, raising the attractive hypothesis that it might be a common pathogenetic denominator in all forms of this syndrome. Although the function of hemojuvelin is unknown, hepcidin levels are depressed in persons with *HJV* mutations,²⁶ suggesting that hemojuvelin is probably a hepcidin modulator. Given the phenotypic severity of *HAMP*- and *HJV*-related hereditary hemochromatosis, it probably plays an important role in this sense. Loss of hemojuvelin-mediated regulation might preclude enhanced hepcidin synthesis in response to increasing plasma iron, with results resembling those caused by loss of hepcidin itself.

No data on hepcidin levels in *TfR2*-related hereditary hemochromatosis have been reported. Its putative role in the uptake of iron by hepatocytes is

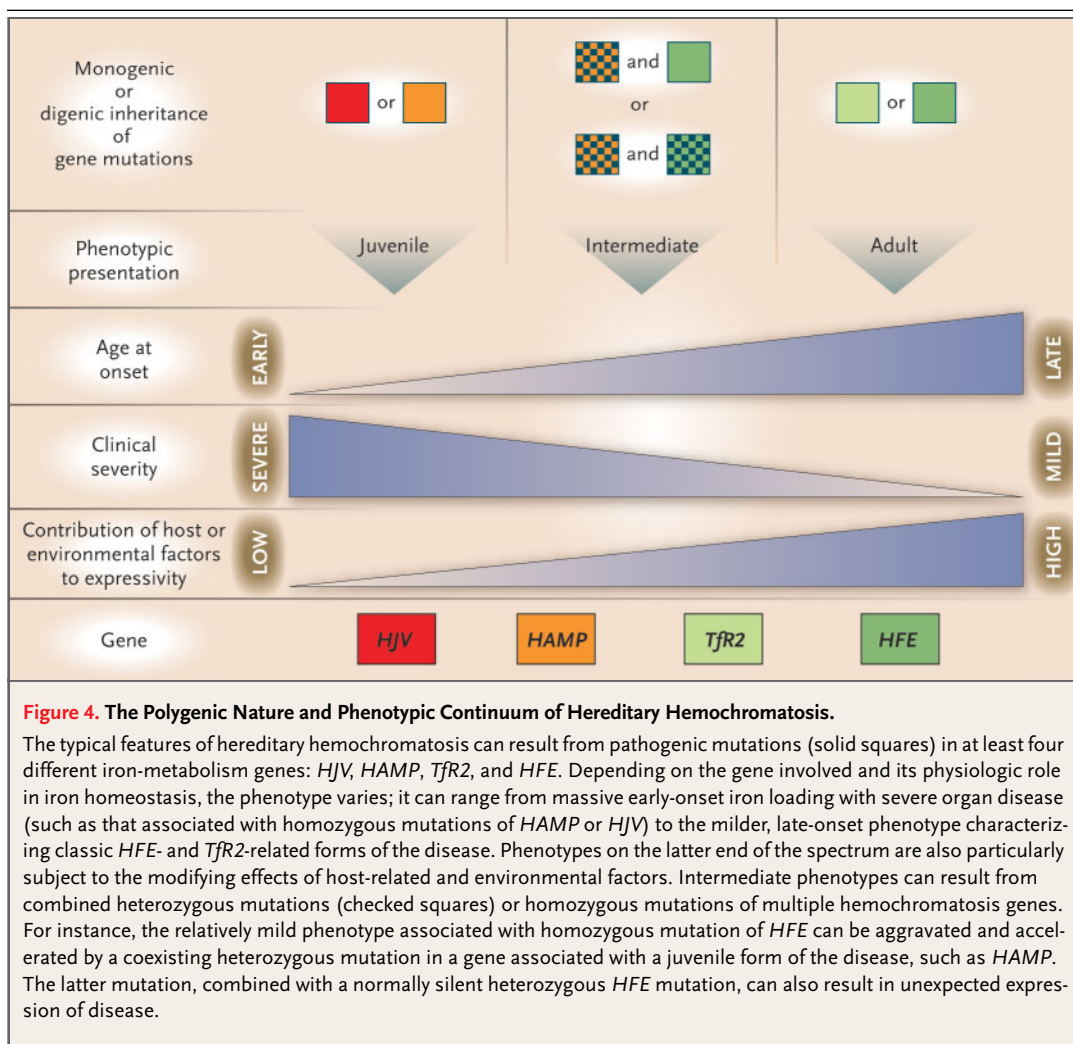
difficult to reconcile with the hereditary hemochromatosis phenotype observed in humans with pathogenic *TfR2* mutations¹⁷ and in *TfR2*-knockout mice.⁶⁷ Although the role of HFE and its interaction with *TfR2* are still controversial,^{39,41,68} these two molecules might be independent but complementary modulators of hepcidin activity. If so, the loss of one might be partially compensated for by the presence of the other, and in the presence of normal *HAMP*, some hepcidin up-regulation could thus be achieved.

The scenario depicted above also allows intriguing speculation on more subtle differences that could result from various combinations of gene mutations in hereditary hemochromatosis (Fig. 4). An increased risk of clinically expressed disease has already been documented in persons with heterozygous mutations in both *HFE* and *HAMP*.⁶⁹ Reports of uncharacteristically severe disease in persons who apparently have *TfR2* mutations, either alone or in combination with *HFE* variants,^{18,70} might also be explained by undetected mutations of other hereditary hemochromatosis genes. The variety of genotypes that can lead to a hereditary hemochromatosis phenotype highlights the importance of defining and classifying this disease as a unique clinicopathologic entity.

DIAGNOSIS, MANAGEMENT, AND SCREENING

Thanks to increasingly early diagnosis, the classic triad of cirrhosis, bronze skin, and diabetes is now rare in adult-onset hereditary hemochromatosis. The most common symptoms at presentation in middle-aged adults are now fatigue, malaise, arthralgia (sometimes associated with hepatomegaly or slightly increased aminotransferase levels). In addition, patients commonly present with increased transferrin-saturation values, which are sometimes found even in the absence of symptoms. Increasing serum ferritin levels herald iron accumulation in the tissues, and values above 1000 ng per milliliter may indicate underlying liver cirrhosis in persons homozygous for the C282Y mutation, regardless of their age or serum liver-enzyme levels.^{71,72} A proposal for the diagnostic workup of these patients is summarized in Figure 5.

For young adults with signs of juvenile-onset disease (hypogonadotropic hypogonadism or unexplained heart failure), the biochemical workup is identical. First-line genetic testing in these cases,



however, consists of *HAMP* and *HJV* sequencing (Fig. 5); since these tests are not widely available, the diagnosis may have to be based on liver-biopsy findings. In young patients with symptoms who have high serum ferritin levels, the hepatic iron index (which is calculated as the hepatic iron concentration [in micromoles per gram of liver, dry weight] divided by the patient's age [in years]) will invariably be higher than 1.9, whereas normal values are less than 1.0.

Once a diagnosis of hereditary hemochromatosis (adult- or juvenile-onset) has been established, further clinical workup is necessary to quantify the iron overload, define its possible visceral or metabolic consequences, and identify risk factors for progression.^{73,75} Family members, particularly siblings, should undergo biochemical testing. Genetic testing is also advisable if a pathogenic mutation

has been identified in the proband, particularly if the proband has juvenile-onset disease.

Patients who have symptoms generally require phlebotomy. Phlebotomy may be deferred in some patients with adult-onset disease who have fairly normal liver function, but it is mandatory when the serum ferritin level is more than 1000 ng per milliliter, because of the risk of underlying hepatic fibrosis. Phlebotomy is the safest, most effective, and most economical therapeutic approach. Initially, 1 or 2 units of blood (each containing approximately 200 to 250 mg of iron) are removed weekly until the serum ferritin level is less than 50 ng per milliliter and the transferrin saturation drops to a value below 30 percent. Attainment of these goals may, in some cases, require up to two to three years. Less aggressive, life-long maintenance therapy is then mandatory to keep the transferrin-saturation value

below 50 percent and the serum ferritin level below 100 ng per milliliter. Initiated early, this regimen can prevent organ damage and improve survival.^{74,76} Established cirrhosis, hypogonadism, destructive arthritis, and insulin-dependent diabetes cannot be reversed, but their progression can be slowed.

Population screening for HFE-related hereditary hemochromatosis is theoretically very attractive. The disorder is highly prevalent and potentially fatal if untreated; safe, effective, and relatively low-cost treatment is available; it can be detected, simply and inexpensively, by measurement of the transferrin saturation. Values greater than 45 percent are an indication for genetic testing.⁷⁷ However, the anticipated benefits (early treatment and prolonged survival) must be weighed against the potentially negative repercussions — psychological, social, and legal — of the diagnosis,⁷⁷ especially in the absence of actual disease.

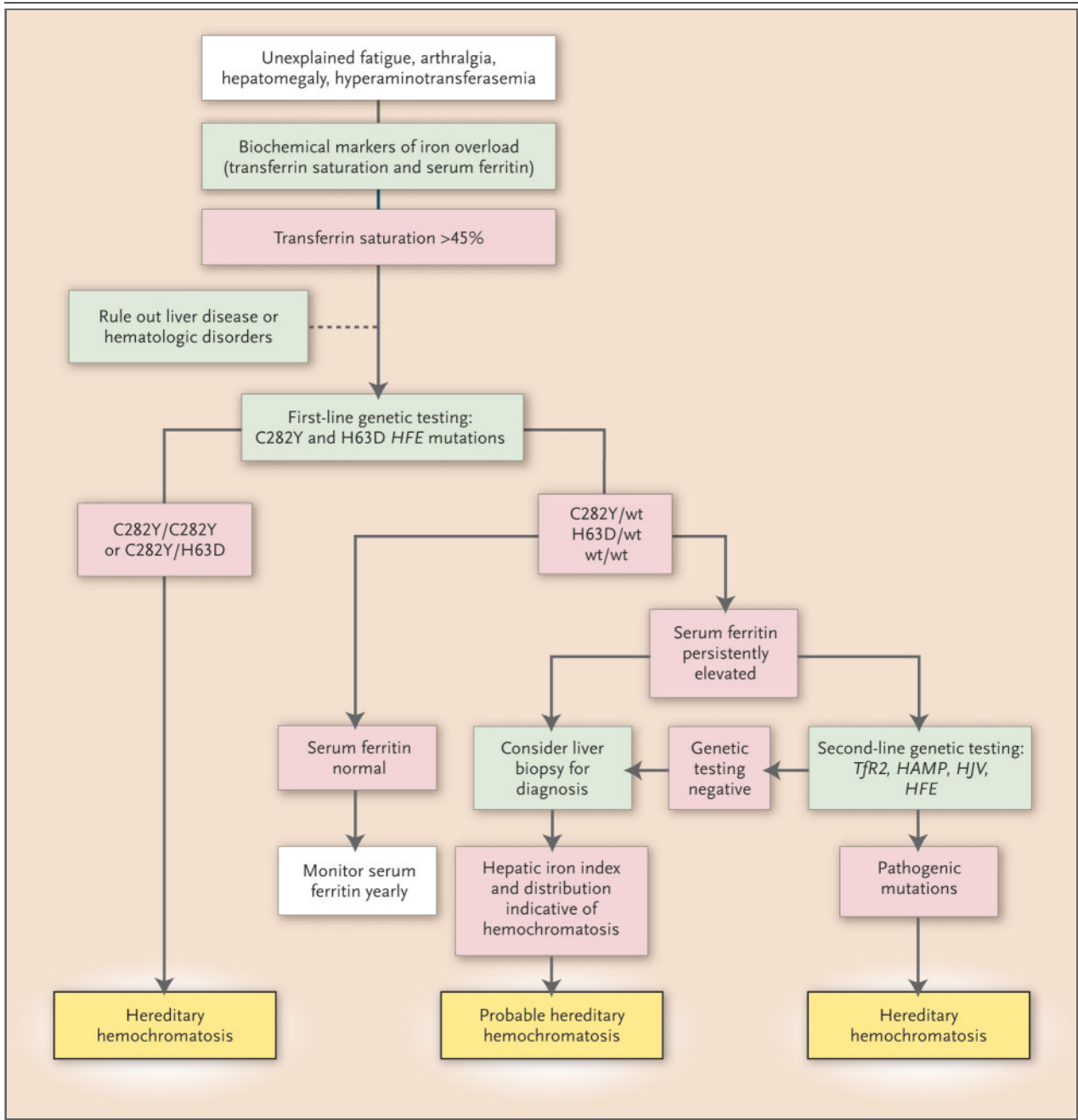
The clinical penetrance of C282Y homozygosity is difficult to define in part because of the numerous factors unrelated to the *HFE* gene, including environmental factors, that can influence its expression (Fig. 2). In addition, how should “penetrance” be defined? Should it be defined in terms of organ damage in general or only as organ damage associated with symptoms, and for which organs? And what about “biochemical” penetrance (i.e., abnormal transferrin-saturation values or serum ferritin levels)? Controversy has also arisen over ascertainment biases related to the health status of populations screened, since health status also influences current estimates of disease prevalence.⁷⁸⁻⁸¹ Studies may be conducted in blood donors, for example, but those populations are preselected for good health and youth, whereas autopsy studies are associated with an opposite bias, and between these two extremes lie numerous other stumbling blocks.^{82,83} General population screening of Norwegian and Australian adults 20 years of age or older has revealed hepatic fibrosis and cirrhosis in 10 percent and 25 percent, respectively, of persons homozygous for the C282Y mutation.^{84,85} Incidence rates for these conditions among adult homozygous relatives of persons with classic hereditary hemochromatosis in the United States were 24 percent among men and 6 percent among women.⁸⁶ During a screening program conducted in a health appraisal clinic, classical multiorgan disease was detected in only 1 of 152 homozygotes on the basis of participants’ responses to a health-status questionnaire, their laboratory-tests results, and findings on phys-

Figure 5 (facing page). Diagnostic Workup of Suspected Adult-Onset Hereditary Hemochromatosis.

In the presence of symptoms and signs such as those shown here, biochemical markers of iron overload should be checked. Hereditary hemochromatosis should be suspected when the transferrin saturation during fasting is above 45 percent (or above 35 percent in premenopausal women) on at least two consecutive occasions, even if the serum ferritin level is normal. When other possible causes of the elevated transferrin saturation (e.g., high serum iron levels due to hepatic cytolysis or low transferrin levels due to liver failure) have been ruled out, first-line genetic testing for the C282Y and H63D mutations of *HFE* (now readily performed) should be considered. A C282Y/C282Y or C282Y/H63D genotype confirms the diagnosis of hereditary hemochromatosis. Second-line genetic testing should be considered for patients with other *HFE* genotypes and persistently elevated serum ferritin levels. Those with normal (i.e., wild-type [wt]) *HFE* may have pathogenic mutations in *TfR2*; those with simple heterozygosity (C282Y/wt or H63D/wt) may have rarer *HFE* mutations or an additional heterozygous mutation in some other iron-related gene. Testing of this type is less widely available and more costly than basic *HFE* evaluation. An alternative approach (widely used for diagnosis in the era before the discovery of *HFE*) is liver biopsy, which can also be performed if second-line genetic tests are negative. In the absence of other obvious causes of iron overload (e.g., post-transfusion siderosis), biopsy findings of parenchymal iron distribution, with a periportal-to-central gradient, and a hepatic iron index above 1.9 (where this index is calculated as the hepatic iron concentration [in micromoles per gram of liver, dry weight] divided by age [in years]) are strongly suggestive of hereditary hemochromatosis. Given the highly variable clinical course of adult-onset disease, however, this cutoff value cannot be considered absolute.^{73,74}

ical examination⁸⁷ (although none underwent a liver biopsy or radiologic examination). Overall, the clinical expressivity of C282Y homozygosity appears to be much lower than previously thought, and the cost effectiveness of screening has been challenged, since many people must be screened in order to prevent severe disease in only a few.

Consensus is greater regarding the value of transferrin-saturation screening in selected populations of patients, such as those with chronic liver disease, type 2 diabetes, arthritis, or impotence. The same practice could easily be extended to all patients seen in primary care settings. The rationale is similar to that for routine cholesterol screening and the prescription of dietary restrictions and drug therapy when risk is verified in the absence of signs of cardiovascular disease. When the results of iron-



metabolism tests are abnormal, clinical biochemical values should be monitored; if genetic tests are positive for hereditary hemochromatosis, prophylactic iron depletion should be considered.

OTHER HEREDITARY IRON-OVERLOAD DISORDERS

The basic features shared by iron-overload disorders associated with mutations in *HFE*, *TfR2*, *HAMP*,

or *HJV* indicate that they are genetic variants of the same syndrome. In all other currently recognized forms of primary iron overload, one or more of these features is missing.

Ferroportin-associated iron overload, currently classified in the OMIM database as hereditary hemochromatosis type 4, was clinically recognized in 1999⁸⁸ and was linked in 2001 to the *SLC40A1* gene, which encodes ferroportin, a protein involved in cellular iron export.^{89,90} Considering the number of

reports published since its discovery, ferroportin seems to be a frequent hereditary cause of hyperferritinemia. The differences between ferroportin-associated iron overload and hereditary hemochromatosis are shown in Table 1. Absence of ferroportin activity leads to abnormal retention (and accumulation) of iron, predominantly by reticuloendothelial macrophages in the liver and spleen and to a lesser extent by hepatocytes and possibly enterocytes. This inappropriate cellular sequestration tends to diminish, rather than augment, the plasma iron pool, reducing the availability of iron for transferrin binding and delivery to the bone marrow. This feature explains why transferrin-saturation values are low or normal during all but the late stages of the disorder; it also explains the borderline iron-deficiency anemia seen particularly in postmenarchal girls and persons undergoing phlebotomy therapy. Most cases reported thus far have been phenotypically milder than those involving classic hemochromatosis, possibly because the organ-damaging potential of reticuloendothelial iron deposition is lower than parenchymal iron deposition. The key to early diagnosis of the disorder is measurement not of transferrin-saturation values but of serum ferritin levels: they may be elevated as early as the first decade of life and often remain high, despite phlebotomy. Aggressive phlebotomy can provoke or aggravate anemia, so transferrin-saturation values and hemoglobin levels must be closely monitored. Ferroportin-related iron overload has been documented in numerous ethnic groups, and ferroportin is probably involved in other iron-overload disorders, including one found in sub-Saharan populations (previously attributed exclusively to dietary excess)⁹¹ and one reported in Melanesians.⁹²

Much rarer disorders caused by mutations of other genes primarily involved in iron homeostasis cannot be adequately covered here, but they also seem to be distinct from the hereditary hemochromatosis syndrome. Aceruloplasminemia,^{93,94} for example, involves a loss of plasma ferroxidase activity, which (like the loss of ferroportin activity) impairs cellular iron efflux, sometimes provoking hypochromic microcytic anemia. Iron accumulates in various organs, including the liver, but brain involvement predominates, and the presentation almost invariably involves neurologic abnormalities. Aceruloplasminemia differs from Wilson's disease, in which hypoceruloplasminemia, when present, is secondary to a copper-transport defect in the liver.

As for atransferrinemia or hypotransferrinemia,⁹⁵ it dramatically impairs plasma iron transport and the delivery of iron to the bone marrow. Its main feature is severe anemia (not surprisingly); iron overload in the tissues results from compensatory increases in intestinal iron absorption. Excess tissue iron has also been attributed to a mutation in the regulatory region of H ferritin,⁹⁶ but this single observation awaits validation. Finally, there is the combination of massive hepatic iron loading and perinatal liver failure that is often referred to as "neonatal hemochromatosis" and is usually fatal.^{97,98} Its hereditary nature is uncertain, although familial cases have been described.

CONCLUSIONS

Hereditary hemochromatosis was once considered a monogenic disorder characterized by excess tissue deposits of iron that inevitably caused organ damage. This view has been shattered by the identification of similar phenotypes associated with mutations in at least four different iron-metabolism genes and by a growing appreciation of the multifactorial nature of the disease. However, the polygenic nature of hereditary hemochromatosis has perhaps been overemphasized by current nomenclature and classifications.⁸ Division of the disease into subtypes based exclusively on genetic criteria can be misleading in clinical settings, since such an approach obscures the unifying clinical and pathophysiological features that define hereditary hemochromatosis. These features include early progressive expansion of the plasma iron compartment, apparently caused by inappropriate iron release by enterocytes and macrophages; progressive parenchymal iron deposits with the potential for severe organ damage; unimpaired erythropoiesis; and an optimal response to therapeutic phlebotomy. The underlying mutations are largely responsible for variations on this theme, and some phenotypes are more subject than other phenotypes to the influence of host-related and environmental factors. However, these variations occur within the boundaries of a unique clinicopathologic syndrome, and that overarching syndrome should always remain uppermost in the mind of the clinician.

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