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FACTORS INFLUENCING DISEASE EXPRESSION IN HEMOCHROMATOSIS

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ABSTRACT

Genetic hemochromatosis is one of the most common inherited disorders in Caucasian populations. The disease frequency in Caucasian populations in Australia, Europe, and the United States is 1:300–400. The basic genetic defect remains unknown, although the hemochromatosis gene is closely linked to histocompatibility antigen (HLA) A, thus allowing early diagnosis in members of affected families. Many factors—environmental, genetic, and nongenetic in nature—influence the degree of iron loading in affected individuals. In particular, pathologic and physiologic blood loss and blood donation influence iron stores in hemochromatosis. The iron concentration in the liver is an important determinant of survival because a hepatic iron concentration in excess of 400 μmol/g dry weight is usually associated with cirrhosis. Patients with cirrhosis secondary to hemochromatosis are at risk of heptocellular carcinoma and complications of portal hypertension. The combination of improved awareness of the condition and the use of HLA typing to identify affected family members has led to earlier diagnosis and therapy, and to an improvement in overall survival.

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INTRODUCTION

Iron is a fundamental requirement of all living organisms, and appropriate iron balance is obligatory for survival. Iron deficiency is a leading cause of morbidity and mortality throughout the world (12). Excessive iron is also detrimental, as evidenced by genetic hemochromatosis. Genetic hemochromatosis is an inherited disorder of iron metabolism in which an inappropriate increase in intestinal iron absorption results in deposition of iron, predominantly in parenchymal cells, with eventual tissue damage and function impairment of the organs involved, especially the liver, pancreas, heart, and pituitary. As a result of presymptomatic diagnosis, the presence of tissue injury is no longer regarded as an essential component for the diagnosis. The fundamental diagnostic feature of genetic hemochromatosis is an increase in total body iron, with predominantly parenchymal cell deposition (13). Thus, the body must maintain a delicate homeostatic control of iron balance to ensure that body iron stores are maintained at an optimal level. Environmental and genetic factors, however, are known to influence this homeostatic control and, thus, the clinical expression of hemochromatosis.

NORMAL IRON PHYSIOLOGY

The body iron content of a healthy adult male is approximately 90 μmol (5 g). Females, particularly those in child-bearing years, contain less iron than males. Approximately 80% of body iron is present in hemoglobin, myoglobin,

and tissue enzymes. The remainder is stored iron, approximately one third of which is in the liver. Storage iron exists in two forms: in the core of the large protein, ferritin; or as hemosiderin, which consists of aggregates of iron and ferritin-derived protein.

In healthy people, iron homeostasis is maintained by regulation of iron absorption in the small intestine. Total body iron content and erythropoietic activity are the most important factors in the physiologic regulation of mucosal iron absorption. Despite extensive research, the mechanisms that regulate iron absorption are still poorly understood. Available data suggest that the intestinal cell is the ultimate controller of iron uptake and transport, but it is possible that circulating messengers that relay information concerning body iron status and degree of erythropoietic activity also play a role.

There does not appear to be a physiologic mechanism for the excretion of excess iron in the normal human being. The loss of iron from the skin, gastrointestinal tract, and genitourinary tract is approximately 1 mg per day in adult males and 2 mg per day in premenopausal women. In the absence of a major excretory pathway for iron in humans, an increase in iron uptake either by a prolonged increase in iron absorption, as occurs in genetic hemochromatosis, or from the administration of parenteral iron must produce an increase in body iron stores, unless there is a concomitant pathologic increase in iron losses.

Iron is absorbed as heme and nonheme iron. Heme iron is better absorbed than nonheme iron; it also improves the absorption of nonheme iron in the diet. The mechanisms of these interactions are not understood. Nonheme iron accounts for over 90% of total iron in the average western diet, and highly refined foods contain approximately 90 μmol (5 mg) per 1000 calories. Normal males consuming 3000 calories per day obtain 270 μmol (15 mg) of iron per day, of which 9–27 μmol (0.5–1.5 mg) is absorbed. The possible range of iron absorption from the normal diet is quite small, extending from less than 9 μmol (0.5 mg) daily in iron-replete subjects to approximately 72 μmol (4 mg) in iron-deficient subjects. The availability of dietary iron is modified by other factors, including dietary fiber, tannins, and drugs such as cholestyramine and tetracyclines, which result in decreased iron absorption, and vitamin C, which increases iron absorption.

Much interest has focused on the possible role of the iron-binding proteins transferrin and ferritin and their membrane receptors in iron absorption. However, recent evidence suggests that neither transferrin nor the transferrin receptor plays a direct role in the absorption of iron, and there are no data on the possible role of a ferritin receptor. In normal subjects, the sloughing of the mucosal cell (which contains iron-rich ferritin) into the gut lumen reduces excessive iron uptake to some extent, but in subjects with genetic iron overload

the role of mucosal cell sloughing is not clear, as the ferritin content of the mucosal cells appears to be lower in hemochromatosis than in secondary iron overload.

Several newly identified iron-binding proteins in the intestine have been described. Peters (48) identified a basolateral membrane-binding site for recently absorbed iron that shows increased binding in vivo following hypoxia, a state known to be associated with increased iron absorption. He suggested that this binding site may be involved in an iron transfer step in intestinal absorption of iron. Conrad et al (16) have reported an iron-binding compound of 56 kDa, whereas Teichmann & Stremmel (70) found evidence for a facilitated transport mechanism mediated by a 54-kDa iron-binding protein located in the microvillous membranes. The role of these proteins in the regulation of normal iron absorption and in disorders of iron metabolism remains to be established.

PREVALENCE OF GENETIC HEMOCHROMATOSIS

Hemochromatosis is now known to be one of the most common autosomal recessive diseases in Caucasian populations. However, early studies suggested the prevalence of hemochromatosis was low. Finch & Finch (31) estimated that hemochromatosis was recognized once in 20,000 hospital admissions and once in 7,000 hospital deaths. Autopsy studies suggested the prevalence was somewhat higher. MacDonald (41) reported that evidence of hemochromatosis was found in 0.2% of autopsies performed, and a similar estimate was made by MacSween & Scott (42). A more recent study by Lindmark & Eriksson (40) in Sweden found hemochromatosis to be present in 0.1% of males autopsied.

Histocampatibility antigen (HLA) typing within families of patients with hemochromatosis suggested that the prevalence of the disease was considerably higher than was previously supposed. The estimated prevalence of hemochromatosis was 0.5% in Utah (21), 0.79% in Australia (7), 0.3% in Canada (11), and 0.3% in Brittany (9).

Population-based studies have confirmed the high prevalence of hemochromatosis in Caucasian populations. A study of 623 male Swedish government employees 30–39 years old found a prevalence of 0.5% (47). Another study of 10,512 hospital inpatients and blood donors from the same rural area of northern Sweden found a prevalence of iron overload of 0.24% (46). The use of blood donors in such studies may result in underestimates of the prevalence. Studies conducted in South Africa, Italy, and the United States (4, 25, 43, 67) have found a very high prevalence, between 0.22–0.95%. In Australia, there is also a high prevalence, at least among predominantly Caucasian populations. In a survey of 1968 employees of two large corporations, subjects were

screened by measurement of transferrin saturation and serum ferritin concentration (39). In all subjects with elevations of both indices, liver biopsy was performed to confirm whether or not the presence of significant iron overload was present. The prevalence was 0.36% in this population, with no difference between males and females.

Thus, contrary to earlier beliefs, it is apparent that genetic hemochromatosis is among the most common genetic disorders in western countries, i.e. greater than five times more prevalent than cystic fibrosis or alpha-1-antitrypsin deficiency.

GENETIC HEMOCHROMATOSIS: MODE OF INHERITANCE

It is now established that hemochromatosis is inherited as an autosomal recessive trait and that the disease-locus is closely linked to the HLA-A locus of the histocompatibility antigen complex on chromosome 6 (64–66). Simon et al have proposed that hemochromatosis is basically a disease of Celtic peoples, arising from a new mutation in ancestral Celtic groups. This is supported by the occurrence of the disease and, in particular, an "ancestral haplotype" in areas of the world populated by such migration patterns.

HLA typing of first-degree relatives of the proband allows the pattern of inheritance within a family to be determined. Affected siblings of the proband usually have two HLA haplotypes identical to those of the proband and are presumed to be homozygous for the iron-loading gene. Unaffected siblings have either one HLA haplotype identical to the proband (i.e. heterozygous) or neither haplotype identical (homozygous normal). The distribution of haplotypes identical to those of the proband among affected siblings fits the criterion proposed by Thomson & Bodmer (72) for a recessively inherited disease coded by an HLA-linked susceptibility gene with a frequency of at least 0.05, and many studies have confirmed the inheritance as an autosomal recessive.

In Australia, the majority of relatives predicted by HLA typing to be homozygous exhibit clinical and/or biochemical expression of the disease. Of 52 homozygous relatives, as determined by HLA studies, 49 expressed the disease either at first assessment or during a follow-up period of up to 8 years (59). However, expression of the disease may be modified by factors such as physiologic and pathologic blood loss, or malabsorption from other causes, e.g. celiac disease. The clinical expression of disease is observed five to ten times more frequently in males than in females. The disease is rarely clinically evident below age 20 years, although with family screening, asymptomatic subjects with iron overload can be identified, even in young menstruating females, and some severe cases of iron overload have been seen in children—the rare juvenile form of this disorder.

THE PUTATIVE BIOCHEMICAL DEFECT

Despite recent advances, the major unresolved issues regarding hemochromatosis still relate to the site and nature of the primary defect. The candidates for the site of the primary defect are the intestinal cell, the liver, and the reticuloendothelial system. It is also tempting to speculate the hemochromatosis gene involves a generalized iron-transport abnormality. The recent identification of copper transporter defects in the copper loading disorder, Menkes disease (22), and also in Wilson disease lend some support to this hypothesis.

The Intestinal Cell

One universal finding in untreated patients with hemochromatosis is that iron absorption is always inappropriately increased above the expected level, as based on their body iron stores. Even with progressive iron loading, iron absorption remains high. Thus, it appears that the normal regulation of iron absorption is not occurring. Many studies have addressed the potential biochemical mediators of this disorder, and several groups of investigators have examined the transferrin and ferritin protein, and more recently messenger (m)RNA, in the gut of animals with iron overload and also in human subjects with hemochromatosis (2, 6, 52). Available evidence does not favor the involvement of transferrin and the transferrin receptor pathway directly in iron absorption. The presence of the gene for each of these proteins on chromosome 3 is also consistent with their noninvolvement in hemochromatosis. It appears that transferrin receptor mRNA and ferritin mRNA are regulated in concert in hemochromatosis, as in normal subjects. This suggests that it is unlikely that the iron regulatory element–binding protein, which regulates the levels of these two proteins, is functioning abnormally (71). In addition, the gene for this protein is located on chromosome 9, and not 6. Recent mapping and linkage studies would also place at least one of the H ferritin pseudogenes on chromosome 6, centromeric to the hemochromatosis locus, which makes this also unlikely to be a candidate gene for the disease.

The Liver

Factors affecting iron absorption include erythropoiesis and body iron load. This suggests that messengers from sites distant from the small bowel can influence iron absorption. Orthotopic liver transplantation has been used experimentally in animals to resolve the issue of whether or not the central defect in hemochromatsosis lies in the liver. Adams et al (1) transplanted an iron-loaded rat liver into a normal rat and showed a decrease in iron absorption. Hepatocyte loading appeared to be responsible for this effect. Thus, this provides some evidence that the liver transports a factor that, depending on the liver's iron stores, causes an appropriate increase or decrease in iron absorption.

Human liver transplantation has provided further details on the fate of both those hemochromatotic subjects who have been transplanted and those who have inadvertently been given a liver from a previously undiagnosed hemochromatotic. At present, the available data are inconclusive as to whether or not the primary defect is hepatic in origin.

The Reticuloendothelial System

In hemochromatosis, in contrast to secondary iron overload, there is a relative paucity of iron and ferritin to be seen in macrophages in the gut and elsewhere. Investigations have failed to reveal any defect in ferritin synthetic capabilities of these cells or in their ability to take up iron. The possibility of an increased transport of iron out of these cells has also been examined. Fillet et al (29) showed that the early release phase of iron from reticuloendothelial cells was considerably enhanced in patients with hemochromatosis. The mechanism by which this enhanced release occurs has not been elucidated, but it may be related to the basic defect in hemochromatosis.

A Universal Cellular Defect

Overall, studies to date still favor a defect in iron release from cells of the gut and possibly from the reticuloendothelial cells. However, if one examines the liver of patients with hemochromatosis in whom there has been some damage or hepatic process in addition to iron overload (e.g. grossly excessive alcohol consumption), iron is present in Kupffer cells, indicating some capability for reticuloendothelial cell iron storage. Hence, one might conclude that the apparent absence of iron and ferritin from these cells is merely a secondary reflection of the route by which iron enters the cells. Evidence for a universal cellular defect so far remains unconfirmed, although, as stated above, the identification of the copper transporter defects in Wilson Disease and Menkes Disease lends support to this hypothesis.

Reverse genetic approaches proceeding from an accurate chromosomal localization are now being used to help identify the hemochromatosis gene. The elucidation of the basic metabolic defect now awaits the cloning and sequencing of the HLA-A linked gene on chromosome 6.

DETERMINANTS OF CLINICAL AND PATHOLOGIC FEATURES

The Liver

The liver is the site of heaviest iron deposition in hemochromatosis. Clinical features related to hepatic iron deposition include lethargy, fatigue, upper abdominal pain, and hepatomegaly. Features of chronic liver disease (e.g.

palmar erythema, spider naevi, and gynecomastia) and portal hypertension occur in patients with established cirrhosis.

The histologic changes in the liver of patients with hemochromatosis are related to the underlying hepatic iron concentration. Iron is found almost exclusively in the parenchymal cells in a periportal distribution in patients with only mild-to-moderate increases in liver iron concentration (24). However, with increasing iron loading, hepatocytes in zones 2 and 3 become iron-loaded. Even in severe iron loading, a gradient in iron content between the periportal zone and zone 3 is almost always maintained (24). Heavier iron loading may be associated with iron accumulation in Kupffer cells. Progressive accumulation of fibrous tissue eventually results in a mixed macromicronodular cirrhosis. In early disease (in the absence of coexistent alcohol injury or viral hepatitis), cell necrosis and inflammation are rare. Concomitant alcohol abuse does not alter the total liver iron concentration. However, alcohol may modify the cellular distribution of iron such that macrophage iron is increased. Patients with hemochromatosis who have sustained alcohol abuse or chronic viral hepatitis may develop cirrhosis earlier in the course of the disease than would otherwise be expected.

The pathogenesis of the fibrotic response to chronic iron overload is poorly understood. Recent advances in cellular and molecular biology have conclusively shown that activated lipocytes (also known as stellate cells or Ito cells) are the hepatic cells responsible for increased collagen production in animals with hemochromatosis (50, 63). Lipocytes exist in normal liver in a quiescent state. However, in certain pathologic conditions they alter their phenotype. These activated lipocytes assume a myofibroblastic appearance, synthesize collagen, and produce alpha-smooth muscle actin (33). These three phenotypic changes have been identified in lipocytes isolated from rats fed a diet enriched with carbonyl iron (50, 63). Alpha-smooth muscle actin expression has been identified by immunohistochemistry in liver sections obtained from human hemochromatosis subjects, and the intensity of the immunostaining for alpha-smooth muscle actin increased with increments in hepatic iron concentration (62). Furthermore, alpha-smooth muscle actin expression is markedly reduced following venesection therapy (62).

Pathologic conditions that cause hepatic cirrhosis are usually associated with a significant hepatic inflammatory infiltrate, and certain elements of the inflammatory response (e.g. cytokines such as platelet-derived growth factor and transforming growth factor-beta) are thought to be essential for activation and the fibrogenic response of the lipocyte (53). Hemochromatosis is atypical because hepatic cirrhosis develops in the absence of significant hepatic inflammation. Nevertheless, it has been postulated that Kupffer cells are activated following phagocytosis of necrotic hepatocytes and subsequently release cytokines, which are important in lipocyte activation and fibrogenesis.

The role of products of lipid peroxidation in lipocyte activation in hemochromatosis is not fully established. It has been postulated that parenchymal cell necrosis may activate lipocytes and that this activation could be mediated by lipid peroxides. Increased formation of lipid peroxides has been shown in livers obtained from carbonyl-loaded rats (3). These lipid peroxidation byproducts may be important mediators of hepatic fibrosis in iron overload, and the recent finding of the abrogation of the fibrogenic response by the addition of dietary vitamin E in animals with iron overload supports this concept (51).

The recent observation that lipocytes in early culture express receptors for tissue ferritin and internalize the ferritin supports the theory of a direct profibrogenic effect of iron on lipocytes (61). Ferritin receptors allow lipocytes to acquire iron, because tissue ferritin acts as a transport protein in the movement of iron between different hepatic cell populations. It is uncertain if the direct effect of iron is mediated only through increased collagen gene expression because, in animals with iron overload, results are conflicting as to whether or not collagen gene expression is increased in the absence of inflammation. It must be emphasized that the ability to induce fibrogenesis in animals with iron overload is variable. Thus, the role of ferritin and the ferritin receptor in iron-mediated hepatic fibrogenesis have yet to be fully established.

Factors Influencing the Development of Hepatocellular Carcinoma

The relative risk for the development of hepatocellular cancer in hemochromatosis is greater than 200 (15). Of patients who present with cirrhosis at the time of diagnosis, approximately 30% will die of hepatocellular cancer (15, 44). The strongest risk factors for its development are being male and the presence of cirrhosis (23). The association with cirrhosis is very strong, although in a small minority of cases hepatocellular cancer can develop in a liver showing only fibrosis (23, 27).

Deugnier et al (23) identified chronic alcoholism and tobacco smoking as additional factors that led to a further increase in the risk for the development of hepatocellular cancer. This study did not identify hepatitis B or previous androgen therapy as important risk factors in the population studied. In contrast, the study of Fargion et al (27) found the presence of HBsAg to be an important risk factor in an Italian population. Hepatitis C was not identified as a significant risk factor for hepatocellular cancer in patients with hemochromatosis. The study of Fargion et al (27) confirmed that alcohol abuse was a risk factor. In patients with cirrhosis, age over 55 years increased the relative risk of hepatocellular cancer 13.3-fold, the presence of HBsAg increased it 4.9-fold, and alcohol abuse increased it 2.3-fold. The development of malig-

nancy is suggested by worsening lethargy or deterioration in hepatic function, or by the development of ascites, abdominal pain, or weight loss (23).

In at-risk patients (i.e. males with cirrhosis over 55 years of age), active surveillance in the form of regular ultrasound examination and alpha-fetoprotein measurements is warranted to detect hepatocellular cancers at an early stage, when treatment is more effective. However, survival of patients with hepatocellular cancer complicating hemochromatosis is poor despite the widespread use of surveillance programs and the recent advances in therapeutics and hepatic surgical techniques. Metachronous tumors are common, and it is feasible to offer transplantation in suitable patients in whom small hepatocellular cancers are detected.

Pancreas

Microscopic examination of the pancreas in patients with advanced hemochromatosis often shows heavy deposits of hemosiderin, principally in the acinar cells. Chemical determination of iron concentration following acid digestion has confirmed avid accumulation in the pancreas with iron concentrations in excess of 600 μmol/g dry weight. In individual patients, in general, pancreatic iron concentration is less than hepatic iron concentration. Dense pancreatic fibrosis may develop and occurs at a tissue iron concentration similar to that at which liver fibrosis is seen (18).

Diabetes mellitus develops in 30–60% of patients with advanced disease. Direct damage to pancreatic acinar cells by deposition of iron and insulin resistance associated with cirrhosis both contribute to the high frequency of diabetes mellitus. Approximately 50% of patients with diabetes will be insulin dependent.

Heart

Electrocardiographic evidence of cardiac dysfunction has been reported in up to 30% of patients with hemochromatosis (57). Iron may be deposited in cardiac muscle to cause cardiomyopathy or cardiac arrythmias. Histologic examination will often show hemosiderin in the heart muscle fibers and in conducting tissue. Myocardial fibrosis is very rare. Cardiac complications are a relatively frequent cause of presentation in young adults and the condition may be misdiagnosed if the disease is not considered. The clinical picture is often that of dilated cardiomyopathy, although restrictive defects and diastolic dysfunction have been documented. The cardiac iron concentration is generally much less than either the pancreatic or hepatic iron concentration. Indeed, it is unusual for the cardiac iron concentration to exceed 100 μmol/g dry weight (18, 45). Occasionally, there is significant regional variation in cardiac iron

concentration (i.e. subepicardial vs subendocardial), which has implications for the accuracy of diagnosis by endocardial biopsy.

Arthropathy

Arthropathy associated with hemochromatosis may be one of the earliest symptoms of the disease. It has been reported in up to 50% of symptomatic patients and may predate the development of either diabetes or hepatic cirrhosis. Arthritis of the hands, especially of the second and third metacarpophalangeal joints, is characterized by cystic and sclerotic changes, cartilage defects, and narrowing of the joint cavity. Chondrocalcinosis, which is distinguished by the presence of calcium pyrophosphate crystals, is often present in larger joints, such as the knees. Hemosiderin is abundant in synovial lining cells. The exact mechanism of injury is unknown, although similar pathology occurs in other iron-loading disorders, suggesting that this is a direct effect of iron.

IMPACT OF EARLY DIAGNOSIS ON DISEASE OUTCOME

In patients with hemochromatosis, the development of hepatic cirrhosis is the single most important determinant of patient outcome (Figure 1). The 10-year survival of hemochromatosis patients with cirrhosis approaches 60%—even with adequate venesection therapy. This is in direct contrast with the survival rate in noncirrhotic hemochromatosis patients, which is identical to the general population. In cirrhotic patients, hepatocellular carcinoma and complications from portal hypertension are the most common causes of death (44). Despite the adverse prognostic significance of cirrhosis in hemochromatosis, the mechanisms underlying the development of hepatic fibrosis and cirrhosis in this condition are poorly understood, and there are currently no strategies to reverse cirrhosis once it is established. Consequently, therapeutic strategies for cirrhotic hemochromatosis patients are limited to venesection, symptomatic treatment of portal hypertension, surveillance for hepatocellular carcinoma, and eventually liver transplantation.

In hemochromatosis, there is a slow accumulation of iron throughout the lifetime. In the early stages, iron accumulation is silent, and only when iron stores reach toxic levels does tissue injury occur. Tissue injury itself is an insidious process, and organ-specific symptoms only occur when there has been significant tissue damage. However, the aim of diagnosis must be early detection and therapy to prevent irreversible organ damage. Symptoms in the early stage are nonspecific and include apathy, lethargy, and early fatigue. Increased awareness of the disease, widespread use of biochemical screening

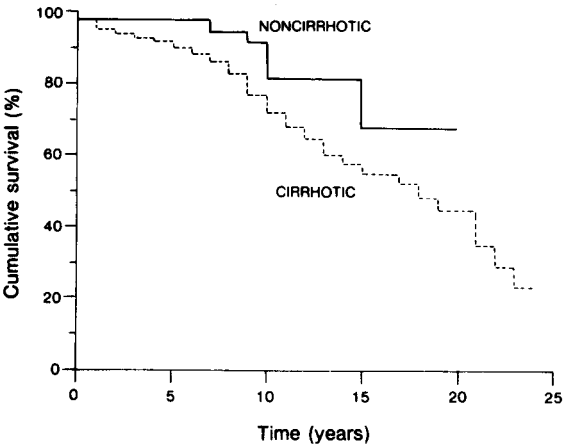

Figure 1 Survival graph demonstrating the influence of cirrhosis on survival in hemochromatosis subjects (from Reference 44 with permission).

tests, screening of asymptomatic relatives, and targeted investigation of high-risk groups, e.g. patients with diabetes mellitus or unexplained arthropathy, have resulted in the earlier diagnosis and therapy of the disease. This has been associated with a dramatic improvement in survival. For example, the cumulative 10-year survival of 83 European patients diagnosed between 1947–1969 was only 66%. Only 5% of these patients were asymptomatic and only 20% were not cirrhotic at the time of diagnosis. In contrast, the cumulative 10-year survival for 84 patients diagnosed between the years 1970–1981 increased to 80%. Approximately 25% were asymptomatic and almost 50% were noncirrhotic. Between the years 1982–1991, the 10-year survival further increased to 91%, and almost 60% were noncirrhotic (69). In this study, as is generally the case, liver cell cancer was the most common cause of death, and all cases of hepatocellular carcinoma occurred in cirrhotic livers. Thus, this study provides conclusive evidence that early diagnosis and therapy is associated with improved survival, and that diagnosis in the early stages will largely prevent the development of associated hepatocellular carcinoma.

In countries with a high carrier rate for hemochromatosis, screening the general population should be considered. Neonatal screening programs similar to those conducted for other inherited diseases await identification of the specific underlying genetic defect. However, the number and frequency of mutations at the hemochromatosis gene locus will determine the feasibility of this approach. Given the high sensitivity and specificity of serum measurements of iron metabolism, such as transferrin saturation, a screening program of adults in the general population is possible (5, 8).

ENVIRONMENTAL FACTORS THAT INFLUENCE BODY IRON STORES IN NORMAL AND HEMOCHROMATOSIS SUBJECTS

The reference range for serum ferritin concentration varies considerably from one population to another. At the lower limit of the reference range, it has been established that serum ferritin concentration of less than 10 μg/liter reflects iron deficiency (35). It is the upper limit of the reference range that is less well defined. For example, a study of low-income men aged 18–45 in Washington State (17) showed the 90th percentile for serum ferritin concentration to be 196 μg/liter, whereas a recent study of asymptomatic Australian men age 30–39 showed the 90th percentile to be 470 μg/liter (38). The reasons for such variation are not fully understood but may relate in part to increased iron intake and meat consumption. It is important to establish an accurate local reference range so that subjects with abnormal iron stores can be accurately identified within the local population.

Environmental Factors

AGE AND GENDER Iron stores vary greatly according to age and gender. Serum ferritin concentration rises in all subjects with age, although the rate of rise in women is slower (17, 38, 73). The levels in women remain low until after the menopause when physiologic iron loss due to menstruation and pregnancy ceases. Thus, it is not suprising that although the prevalence of hemochromatosis is equal in men and women, heavy iron overload due to the disease occurs earlier in life in men than in women.

BLOOD DONATION After gender, the next most important influence on body iron stores in healthy individuals is the frequency of blood donation (30, 38). Frequent blood donation causes a marked decrease in serum ferritin concentration in both men and women. However, there is a greater increase in the prevalence of iron deficiency among women, presumably because most women have lower initial iron stores, which are more easily depleted. Frequent blood donation may be sufficient to delay or even completely prevent the accumulation of excessive iron in individuals with hemochromatosis.

DIETARY IRON INTAKE It is difficult to be certain of the degree to which dietary iron content influences iron stores because most studies have examined single populations in which dietary iron content is relatively uniform. The food that makes the greatest contribution to bioavailable iron in the diet is meat. Variation in meat consumption may contribute to the varying iron stores in different populations. For example, in the United Kingdom and Sweden, where

median serum ferritin is considerably lower than in Australia, meat consumption is only 68% (UK) and 54% (Sweden) that of Australia (32). There is some evidence that within a population meat consumption has some effect on iron stores. In the Australian population, diet had a significant effect on the serum ferritin concentration of women (38). This may be because women must normally absorb a greater proportion of the iron in their diet to maintain iron balance (13). Variations in diet are likely to influence the rate of iron accumulation in hemochromatosis but would be unlikely to totally mask expression of the disease.

ORAL IRON INTAKE Scant data have been published on the effect of continuing oral supplementation once iron deficiency has been corrected. It would certainly be expected to accelerate the onset of heavy iron loading in subjects with hemochromatosis. Whether or not pharmaceutical doses of iron given over many years can overcome the normal barriers to excessive iron absorption and lead to iron accumulation, which mimics hemochromatosis, is unresolved.

PATHOLOGIC BLOOD LOSS AND MALABSORPTION Regular blood loss of more than about 10 ml of blood daily is likely to lead to iron deficiency. Individuals with hemochromatosis can increase their iron absorption to more than 4 mg per day, but even they can become iron deficient if losses are prolonged and severe. Chronic gastrointestinal disease such as inflammatory bowel disease may mask the expression of hemochromatosis in homozygotes for the disease. Malabsorption is a less-common cause of iron deficiency in Western societies. However, individuals with diseases such as celiac disease frequently become iron deficient, and again this could mask the expression of hemochromatosis.

FACTORS THAT MAY AFFECT SERUM FERRITIN CONCENTRATION WITHOUT AFFECTING IRON STORES Serum ferritin concentration does not always accurately reflect body iron stores. It is elevated out of proportion to iron stores in the acute phase response accompanying inflammation or neoplasia (26). Liver disease of any cause is often associated with a high serum ferritin concentration, presumably as a result of leakage of ferritin from damaged hepatocytes because hepatocytes normally contain relatively high concentrations of ferritin (60). The nonspecific association of elevated serum ferritin concentration and liver disease frequently leads to confusion as to whether the patient has hemochromatosis or some other cause of liver disease. Indeed, alcohol was proposed as a causative factor for iron overload. However, it is now established that

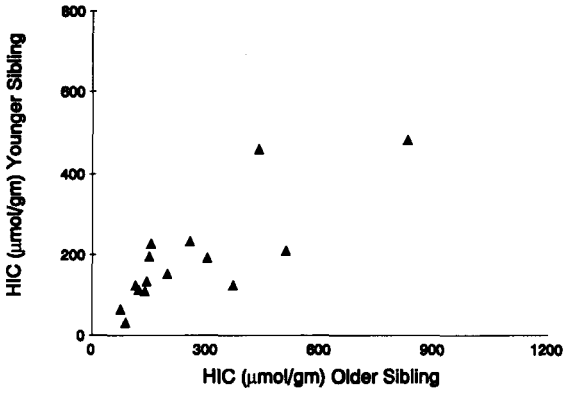

Figure 2 Comparison of hepatic iron concentration between same-sex siblings demonstrating a high degree of concordance (from Reference 19 with permission).

alcohol is associated with an increase in serum ferritin as a result of hepatocellular damage.

Genetic Factors

Increasing evidence suggests that, in many inherited conditions, there is a close relationship between genotype and phenotype. For example, in familial adenomatous polyposis, mutations in exons 3 and 4 produce an attenuated form of the disease, whereas mutations in later exons produce the classic phenotype (68). Similarly, in cystic fibrosis different mutations account, in part, for the variable phenotypic expression of this disease (37). Concordance of disease expression between affected siblings is important evidence that genetic factors are significant determinants of disease expression. In a study of disease expression in siblings with hemochromatosis, Crawford et al (19) provided evidence of a wide variation in hepatic iron stores that could not be accounted for by environmental factors, which may influence body iron stores. Despite this variation, affected siblings of identical sex had similar amounts of liver iron (Figure 2). Of the 22 same-sex sibling pairs studied, the hepatic iron concentration of one sibling was less than 50% of the other in only three pairs. In each of these three pairs, discordance could be explained by multiple previous blood donations or by HLA nonidentity. Thus, these data supported the hypothesis that the extent of hepatic iron loading is principally determined by genetic factors.

The use of multiple, highly polymorphic, genetic markers has provided further evidence of a definitive genotype-phenotype in hemochromatosis. Five

Table 1 Iron indices in hemochromatosis patients according to AH status[a]

Patients	No.	SF (μg/liter)	TS (%)	HIC (μmol/g)	HII (HIC/age in years)
All					
No AH	78	973	80.0 ± 16.6	228 ± 116	5.4 ± 3.2
One AH	37	700	79.7 ± 17.6	189 ± 160	5.0 ± 2.9
Two AH	14	1030	88.4 ± 7.8	302 ± 149	9.1 ± 4.5[b]
Male only					
No AH	53	1000	78.9 ± 15.6	240 ± 157	5.6 ± 3.3
One AH	21	905	80.8 ± 17.5	188 ± 170	5.0 ± 3.1
Two AH	8	1030	89.4 ± 6.2	318 ± 176	10.1 ± 4.6[b]

[a] AH, Ancestral haplotype; SF, serum ferritin (median); TS, transferrin saturation (mean ± SD); HIC, hepatic iron concentration (mean ± SD); HII, hepatic iron index (mean ± SD). (From Reference 20 with permission.)

[b] $P < 0.003$ when compared with the other two groups.

polymorphic markers—D6S248, D6S265, HLA-A, HLA-F, and D6S105—all show significant allelic association with hemochromatosis, and a specific combination of the alleles of these markers forms a common, predominant ancestral haplotype in Australian patients (36). In other inherited diseases, haplotypes have been found to be in linkage disequilibrium with different mutations at the disease-gene locus, and specific mutations have been found to account for the variable phenotypic expression of the disease (49). In a further study of disease expression in hemochromatosis patients, Crawford et al (20) have recently shown that Australian patients who are homozygous for the ancestral haplotype accumulate more liver iron (as measured by hepatic iron index) than affected patients who do not carry two copies of this haplotype (Table 1). A similar finding has been reported in Italian patients in whom the presence of the ancestral haplotype is associated with a homogeneous and severe phenotype (54). Thus, it appears at present that genotype may be an important determinant of phenotypic expression in hemochromatosis.

DISEASE EXPRESSION IN THE HETEROZYGOTE: IMPLICATIONS FOR THE HIGH PREVALENCE OF THE DISEASE

HLA typing of hemochromatosis pedigrees allows identification of putative heterozygote subjects. Data from studies conducted in many centers suggest that heterozygote subjects may exhibit partial phenotypic expression of the disease. This expression is manifest principally by an increase in serum transferrin saturation. In many studies, the mean transferrin saturation in heterozygous subjects is approximately 35%, whereas the mean transferrin saturation

of the general population is approximately 25% (74). However, heterozygotes do not manifest clinical features of the disease. Many authors claim that serum ferritin is often elevated in heterozygous subjects. However, when corrections for age and gender are made, there does not appear to be any significant difference in the serum ferritin concentration between groups of heterozygotes and control subjects. Male heterozygote subjects carrying the ancestral haplotype do not appear to have an increase in transferrin saturation or serum ferritin concentration when compared to heterozygote subjects without the ancestral haplotype. However, the mean serum ferritin of female subjects who carry the ancestral haplotype is approximately twofold that of heterozygote females who do not carry this haplotype. It is of interest that the frequency of iron deficiency appears to be much less in female heterozygous subjects than in the general population (20). This suggests a selective advantage conferred by the presence of one copy of the hemochromatosis gene in protecting women against iron deficiency. Female homozygotes would benefit, in a similar manner, during their reproductive years. Thus, all female groups have a survival and reproductive advantage. Some male homozygotes would be lost because of iron overload, but the majority of male individuals survive to reproductive age. Thus, the selective advantage conferred by protection from iron deficiency may be an important factor determining the prevalence of the disease in the community.

EFFECT OF THERAPEUTIC STRATEGIES ON DISEASE EXPRESSION

Phlebotomy

The treatment of hemochromatosis involves the removal of excess iron as quickly as possible, as well as the treatment of complications such as diabetes mellitus. Iron is most readily removed by weekly or twice weekly phlebotomy of 500 ml until body iron stores return to normal, as indicated by serum ferritin concentration and transferrin saturation. Thereafter, one phlebotomy approximately every 3 months usually suffices to maintain normal iron stores.

A repeat liver biopsy at this stage usually reveals absence of stainable iron in hepatocytes and in Kupffer cells. Moreover, preexisting fibrosis may completely reverse (14). However, cirrhosis is irreversible and the patient is predisposed to primary liver cell carcinoma thereafter. Particularly noteworthy is the development of large macronodules with large areas of preserved parenchyma, such that needle biopsy may suggest normal hepatic architecture (57).

Although no controlled prospective trials of phlebotomy therapy in this disease have been published, two retrospective evaluations have strongly indicated increased survival after phlebotomy, particularly in nonalcoholic sub-

jects (10, 55). With removal of iron by repeated phlebotomy, patients lose their lethargy, the liver decreases in size, biochemical tests return to normal, skin pigmentation decreases, and cardiac failure may be reversed (34). Carbohydrate intolerance improves in 30–40% of patients, resulting in a reduction in insulin requirements or drug therapy. Removal of excess iron has little or no effect on hypogonadism, testicular atrophy, or portal hypertension or arthropathy.

Liver Transplantation

Orthotopic liver transplantation is an effective therapeutic modality for end-stage liver disease due to hemochromatosis, as well as for other liver diseases. Recent reports of a poor outcome following liver transplantation in hemochromatosis (28) probably reflects the late diagnosis of the disease and the lack of therapy before transplantation. Such patients are therefore susceptible to cardiomyopathy and other complications of their increased body iron stores. In contrast to such reports, results from The Australian Liver Transplant Registry would indicate that liver transplantation is required infrequently for this disease, probably because of comparative early intervention and subsequent reversal of the natural history of the disease by phlebotomy. Furthermore, the Australian data indicate that survival of hemochromatosis patients undergoing liver transplantation following intensive venesection therapy is not different from other patients with end-stage liver disease (LW Powell, unpublished observations).

Whether or not the disease recurs in the liver after orthotopic liver transplantation is still unresolved. Until 1991, 22 hemochromatotic patients had been subjected to liver transplantation worldwide and none had shown evidence of significant iron accumulation in hepatocytes up to 5 years after transplantation (56). Subsequent experience has extended these observations, and at the time of writing, there is no well-documented case of significant reaccumulation of iron in such patients.

SUMMARY

The clinical and pathologic features of hemochromatosis are determined primarily by the severity of iron loading, which in turn is affected by many factors of environmental, nongenetic, and genetic origin. Neither the biochemical mediators of the increased iron absorption or the underlying genetic defect in this disease are known. An understanding of the molecular and genetic factors that regulate iron absorption awaits identification of the basic biochemical and genetic defects.

Similarly, the mediators of tissue fibrosis and liver cancer in hemochroma-

tosis are poorly understood. This is unfortunate because the development of hepatic cirrhosis or hepatocellular carcinoma indicates a poor prognosis. To date, the greatest influence on the overall prognosis in hemochromatosis is early diagnosis. Early diagnosis and therapy, either by family or community screening programs, is associated with symptomatic improvement and improved survival and should prevent the development of cirrhosis and associated hepatocellular carcinoma.

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