HLA-H : Final Piece in the Hemochromatosis Puzzle?

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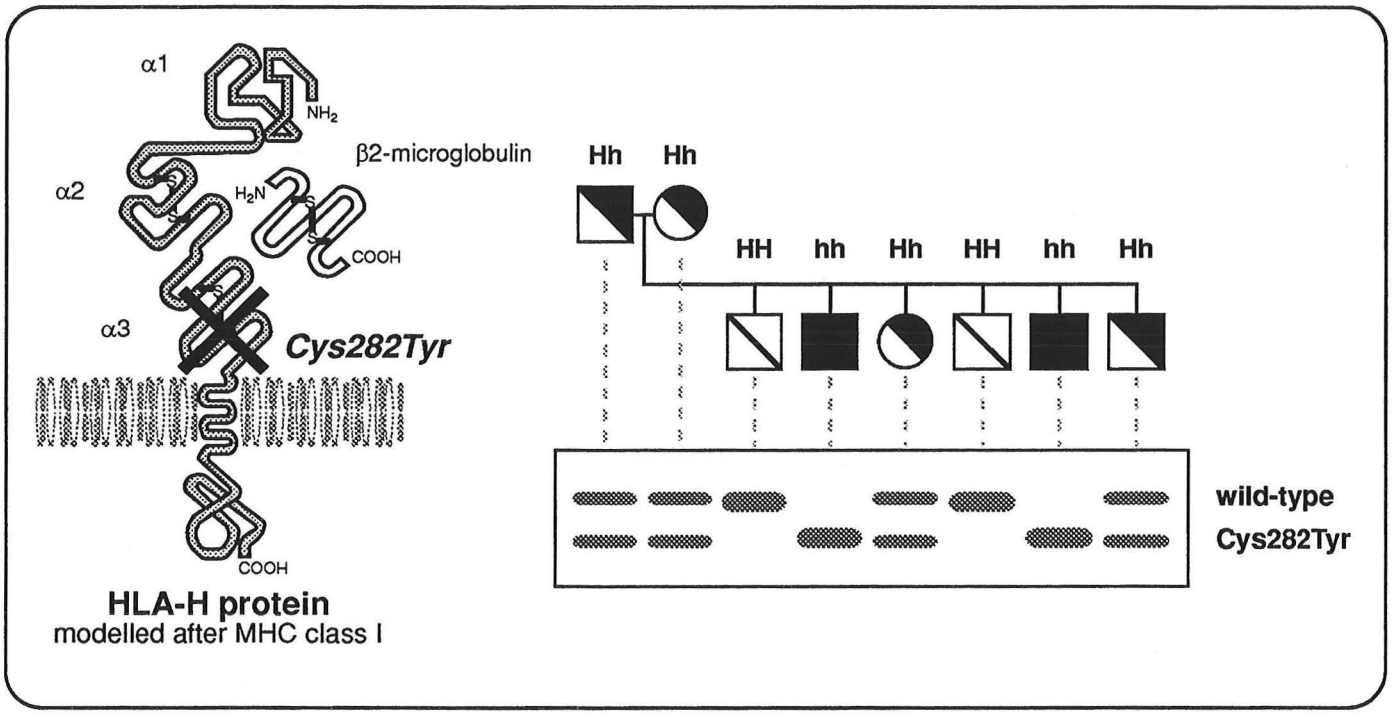

"....when you have eliminated the impossible, whatever remains, however improbable, must be the truth"

Sherlock Holmes [Sir Arthur Conan Doyle]

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Clinical interests: Acute hepatitis, genetic liver disease, ascites

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This contribution concentrates on progress in the understanding of hemochromatosis since it was last reviewed at this venue (James Shorey, M.D., "Genetic Hemochromatosis", February 5, 1987). Original citations for statements not specifically annotated are contained in references 1 and 2.

Case Reports

#1 – A 49 year old woman was referred for evaluation of elevated hepatic enzymes. She had a 20 year history of consuming >6 beers/day and 5 years of arthritis. Physical examination revealed hepatomegaly and dermatitis. Investigations: bilirubin 0.7 mg/ dl, aspartate aminotransferase (AST) 125 IU/L, alanine aminotransferase (ALT) 49 IU/L, alkaline phosphatase (AP) 156 IU/L, γ-glutamyl transpeptidase (GGT) 956 IU/L; viral hepatitis serologies negative. A liver biopsy revealed cirrhosis with extensive iron staining, moderate fatty infiltration, iron content 12,057 μg/g dry wt. Iron studies demonstrated serum Fe 204 μg/dl, total iron binding capacity (TIBC) 272 μg/dl (75% saturation) and serum ferritin 2,255 ng/ml. Diagnosis: cirrhotic hemochromatosis. Within 2 years of diagnosis, she developed complications of cirrhosis (ascites).

#2 – A 67 year old man with chronic demyelinating polyneuropathy was found on routine evaluation to have abnormal iron studies, serum Fe 238 μg/dl, TIBC 252 μg/dl (94% saturation), serum ferritin 802 ng/ml. His hepatic enzymes were entirely normal, AST 16 IU/L, ALT 19 IU/L, AP 46 IU/L as was his bilirubin (0.7 mg/dl). A liver biopsy revealed extensive iron deposition but no fibrosis, hepatic iron content 11,062 μg/g dry wt. Diagnosis: pre-fibrotic hemochromatosis.

These cases illustrate some of the differences in presentation observed in patients with the commonest inherited liver disease encountered in North America and Europe, hemochromatosis (Table I). Both patients almost certainly have human leukocyte antigen (HLA)-linked hemochromatosis (also referred to as primary, idiopathic, hereditary or genetic hemochromatosis in the literature), although no family studies have confirmed an autosomal recessive inheritance linked to the HLA region on chromosome 6.

History of Hemochromatosis

In the second half of the nineteenth century, Trousseau and Troisier recognized the association of cirrhosis with diabetes and pigmentation and von Recklinghausen identified the pigment as iron, coining the term "hämochromatose" since he considered the iron to originate from blood (cited in reference 2). The landmark monograph by Joseph H. Sheldon, a consultant physician and clinical lecturer at the University of Birmingham, reviewing 311 cases of hemochromatosis, was published in 1935. He concluded that a single inborn error of iron metabolism was responsible for multiple organ involvement and that the disease was familial (cited in reference 2). The next major milestone was the linkage of the hemochromatosis gene to the small arm of chromosome 6, close to the HLA-A class I region.[3] Almost 20 years later, the hemochromatosis gene, tentatively termed *HLA-H*, has been identified.[4] This inborn error of iron metabolism is caused by a point mutation in a critical cysteine residue that is predicted to be necessary for the stable expression of an HLA class I-like molecule. The exact function of this molecule is thus far undetermined. The importance of normal iron metabolism, however, in emphasized by the protean manifestations of hemochromatosis as well as its accompanying morbidity and mortality.

Normal Iron Metabolism

Iron (*ferrum*) is the 4th most abundant element in the earth's crust (after oxygen, silicon

Table I: Prevalence of Inherited Liver Diseases

Disease	Homozygote Frequency (q^2)	Mutant Gene Frequency (q)	Heterozygote Frequency (2pq)
Hemochromatosis	1:400*	1:20*	1:10*
α1-AT deficiency	1:1600	1:40	1:20
Cystic fibrosis	1:2500	1:50	1:25
Wilson's disease	1:30000	1:170	1:85

* Caucasian population

From: Powell *et al*, Semin Liver Dis 16:55-63, 1996

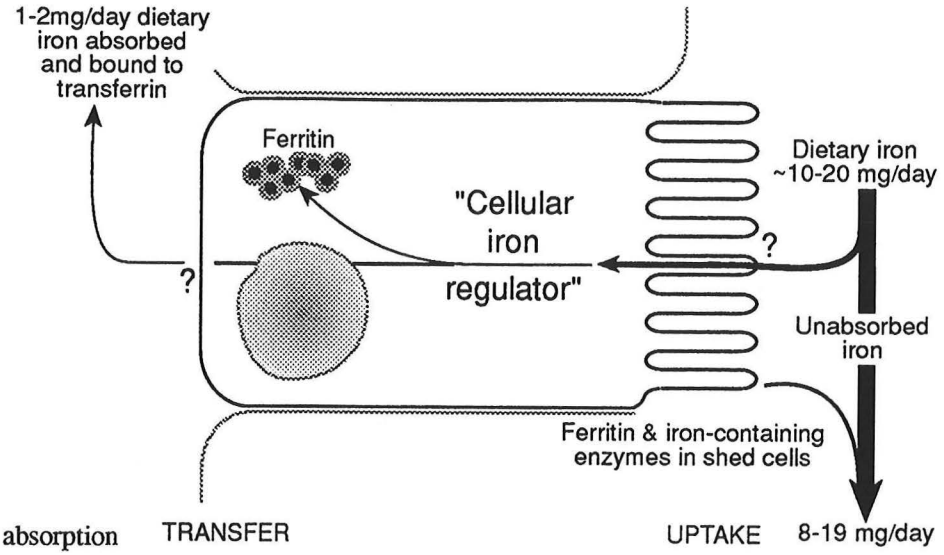


Figure 1: intestinal iron absorption

and aluminum). It is essential for the life of almost all organisms, the few exceptions being confined to strains of *Lactobacillus* and *Bacillus*. The major function of iron is in oxidation-reduction reactions that utilize its alternative states Fe^{2+} and Fe^{3+}.[5] Its capacity to generate reactive oxygen intermediates ($Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2\bullet$ [superoxide anion] and $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\bullet$ [hydroxyl radical]) is considered central to the pathologic effects of iron overload. In man, iron enters the body in the diet, with an average of 6mg Fe/1000 calories and the total body iron content is ~2300mg in women (60kg) and ~3500 mg in men (70kg).[1] Hemoglobin in red cells contains ~60-70% of the total, with the remainder in myoglobin, iron-containing enzymes (heme and non-heme), storage (ferritin and hemosiderin) and transport (transferrin) forms. Protein-bound iron circulating in the plasma in ferritin or transferrin accounts for <2%. The total iron content is normally tightly regulated because accumulation of excess quantities in tissues is deleterious. In addition to the effects of pathologic iron levels on the liver, heart, endocrine glands, joints and skin manifest in hemochromatosis, iron has been implicated in the pathogenesis of ischemic heart disease and malignancy.[6, 7]

Iron absorption:

Total body iron content is regulated by controlling the level of absorption from the diet, via the intestinal epithelial cells, into the remainder of the body.[5] The (in)solubility of iron at neutral pH (Fe^{2+} 10^{-1}M and Fe^{3+} 10^{-18}M at pH 7) favors failure of absorption and, indeed, only ~10% of dietary iron is absorbed. The process of intestinal iron absorption occurs in three phases (Figure 1). In the initial phase, mucosal uptake, iron is transported into the intestinal epithelial cell. Iron (Fe^{2+}) in heme from animal products, released from food during digestion, is transported intact across the microvillus membrane and is more readily

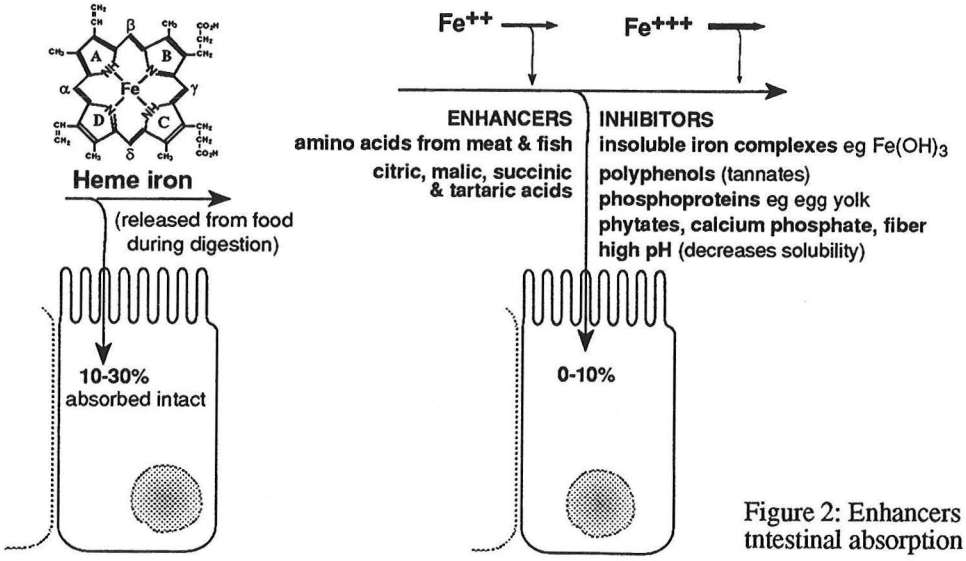
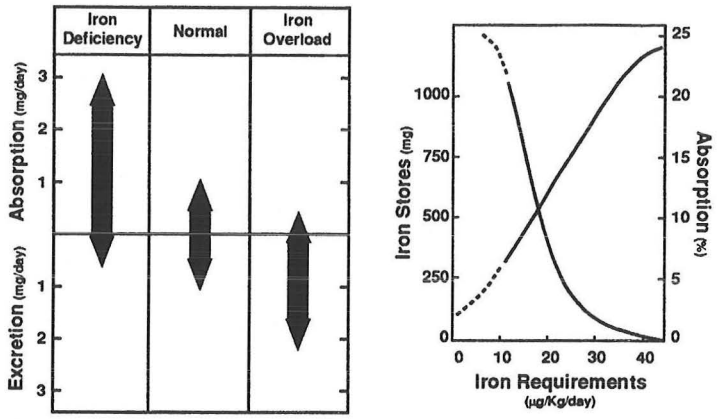


Figure 2: Enhancers and inhibitors of tntestinal absorption of dietary iron

Figure 3: Regulation of Iron Balance by Controlling Absorption



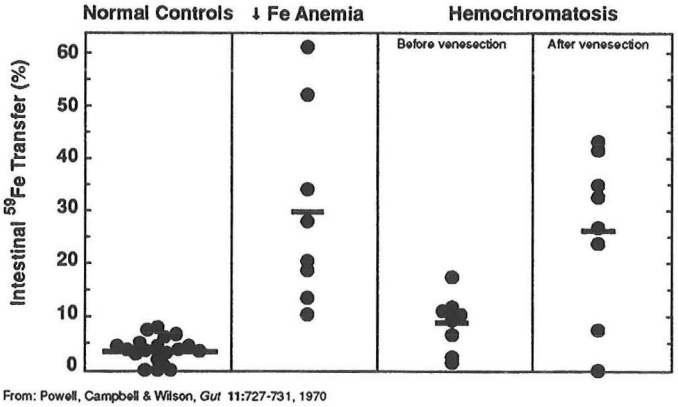
From: Bothwell, Charlton & Seftel, *SA Med J* 39:892-900, 1965; Finch & Huebers, *N Eng J Med* 306:1520-1528, 1982

absorbed (10-30% absorption) than non-heme iron (Figure 2).[1] Only 1-10% of dietary non-heme iron is absorbed. Uptake of non-heme iron can be enhanced by other dietary factors such as amino acids from meat and fish, ascorbate, citrate, sugars and acids (malic, succinic and tartaric) by increasing solubility. Other dietary and intestinal factors inhibit absorption, forming insoluble complexes of iron with tannates (in coffee and tea), phosphoproteins (egg yolk), phytates, fiber and calcium phosphate. Gastric acid, pancreatic bicarbonate, bile salts and intestinal digestive proteases can also alter iron solubility and thereby alter absorption.[1]

The actual process of iron uptake has not been completely characterized. It appears that only Fe^{2+} crosses the membrane, however, the exact mechanism is unknown.[5] After uptake, the iron is part of a "cellular pool" (phase II) and may be stored as ferritin or transported across the basolateral membrane of the cell. The transfer of iron across the basolateral membrane (phase III) completes the process of absorption. Excess iron that is not transported across the basolateral membrane becomes incorporated into ferritin in intestinal epithelial cells, will be excreted in shed cells if not transferred into the body.

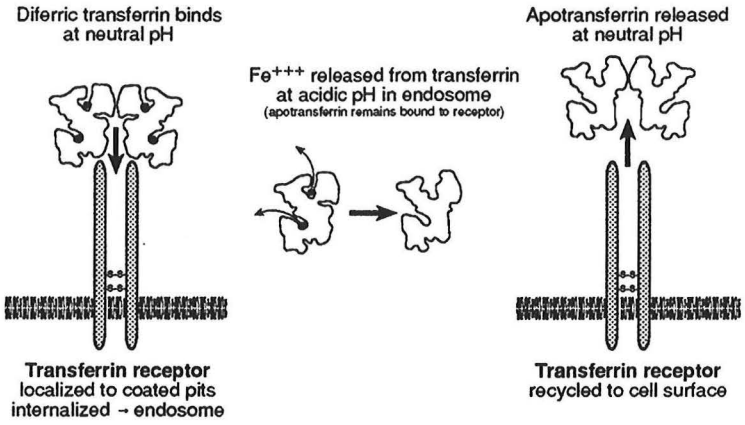
Iron balance is controlled by regulating iron absorption to maintain a normal total body iron content (Figure 3).[8] The factors which regulate dietary iron absorption include iron stores, erythropoietic rate, anemia and hypoxia. When there are no iron stores, absorption rates increase and there is enhanced transfer of iron across the basolateral membrane. When iron stores are high, absorption is decreased. Increases in the erythropoietic rate also increase iron absorption. This is particularly important in disorders characterized by high rates of ineffective erythropoiesis such as thalassemia because increased absorption of dietary

Figure 4: Unregulated Iron Absorption in Hemochromatosis



From: Powell, Campbell & Wilson, *Gut* 11:727-731, 1970

Figure 5: Transferrin—Transferrin Receptor Interactions



iron in these conditions contributes to the iron overload. Anemia and hypoxia both increase iron uptake. In iron deficiency anemia, both uptake and transfer across the basolateral membrane are enhanced. The findings in hemochromatosis are those of inappropriately high absorption of iron (Figure 4, from reference 9).[9, 10] Mucosal uptake of iron is either normal[10] or elevated[9, 11] and there is increased retention of labeled iron in the body, indicating enhanced transfer (phase III).[9, 10]

Cellular iron:

Once absorbed, iron reaches the blood-stream and becomes tightly bound to circulating plasma transferrin.[1, 5] Each molecule of apotransferrin (iron-free) binds two Fe^{3+} at neutral pH to become diferric transferrin (Figure 5). At neutral pH, diferric transferrin binds with high affinity to the transferrin receptor on plasma membranes of erythroid precursors and proliferating cells and is taken up by the process of receptor-mediated endocytosis (Figure 6). Transferrin receptors are present as homodimers in coated pits on the plasma membrane. These structures internalize, becoming coated vesicles and then endosomes. When the pH of the endosome falls, the Fe^{3+} dissociates from the transferrin. The resultant apotransferrin remains bound to the receptor in the acid pH of the endosome. The iron is transferred into the cell, by unknown process(es) and the endosome recycles to the plasma membrane. At the neutral pH of the cell surface, apotransferrin dissociates from the transferrin receptor to complete the cycle. The intracellular iron becomes part of a cellular pool, being incorporated into heme and non-heme iron-containing enzymes and proteins. In erythroid cells, the vast majority of iron is processed into heme in hemoglobin. In other proliferating cells, the iron-containing enzyme ribonucleotide reductase, which is essential for DNA synthesis, is a critical target. Transferrin and transferrin receptors are normal and function normally in HLA-linked hemochroma-

Figure 6: Receptor-mediated Uptake of Iron from Diferric Transferrin

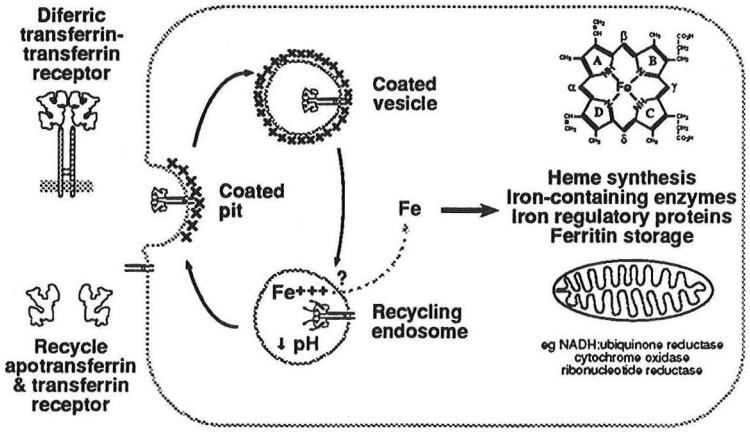
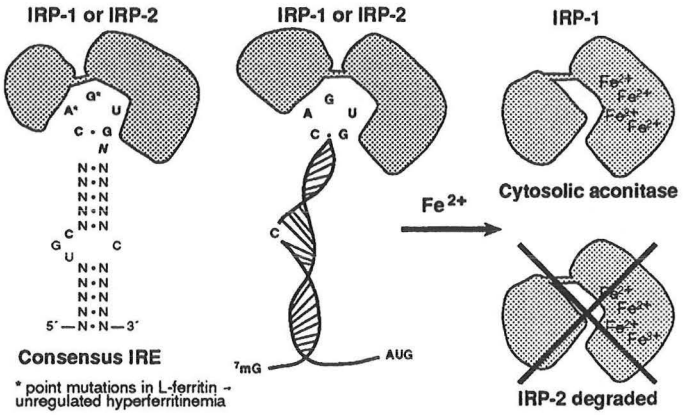


Figure 7: Iron Regulatory Proteins (IRPs) Recognize Iron Response Elements (IREs)



tosis except for the high transferrin saturation with iron.[12-14]

The hepatocyte is in a unique position to acquire iron from transferrin. Iron joins transferrin in the portal venous system and in the sinusoids of the hepatic parenchyma, transferrin has direct access to the plasma membrane of hepatocytes since there are fenestrations in the sinusoidal endothelial cells. Similarly, in the bone marrow, transferrin does not have to traverse an endothelial cell barrier. Consequently, hepatocytes are constantly exposed to circulating iron bound to transferrin. Available data also indicate that hepatocytes may acquire iron from transferrin by a high capacity, low affinity process as well as via receptor-mediated endocytosis.[15] Hepatocytes also preferentially take up non-transferrin bound iron from the portal venous system. The concentration of non-transferrin bound iron rises substantially once transferrin saturation is elevated. Thus, the hepatocyte is at risk for iron overload particularly when there is an increase in iron absorption.

Regulation of cellular iron:

Inside the cell, iron is distributed between heme and non-heme enzymes and proteins, including the iron regulatory proteins (IRPs), IRP-1 and IRP-2.[16] Additional iron is stored as ferritin.[17] IRPs are proteins that regulate intra-cellular iron levels and ensure that any iron, not required for essential enzymes and proteins, is stored as inert ferritin.[16] When cells are iron-depleted, IRPs are iron-poor and they acquire RNA-binding activity (Figure 7). IRPs interact with stem-loop structures in the 5′ or 3′ region of mRNAs, iron response elements (IREs), and thereby alter the ability of the mRNA to be translated or its stability. The individual nucleotides in the loop region of the stem-loop structure are critical for recognition and point mutations prevent regulation.[18, 19] A single IRE is found in the 5′ untranslated region (UTR) of

Figure 8: IRPs are Active in Iron Deficiency States
(↓ storage & ↑ uptake)

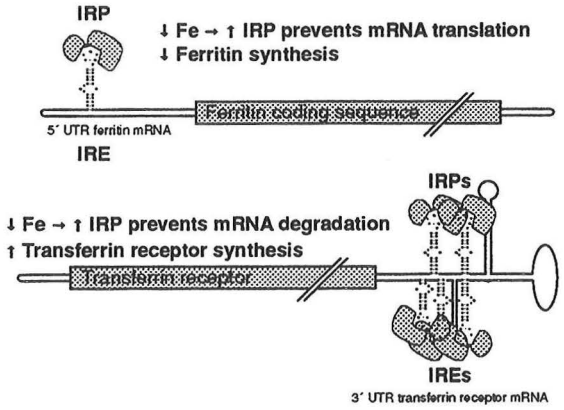


Figure 9: IRP Activity is Absent in Iron-replete States
(↑ storage & ↓ uptake)

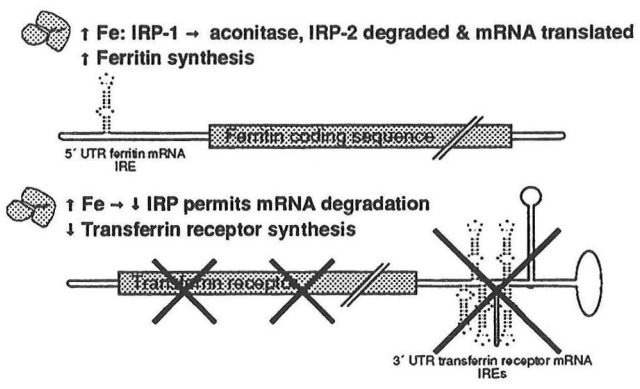


Figure 10: Biological Iron Storage in Ferritin

Ferritin (24 subunits)

Stores iron as inert Fe^{3+} with 6 oxygen atoms/Fe atom

Synthesized intra-cellularly

Receptor-dependent cellular uptake of serum ferritin into hepatocytes

H-ferritin subunit

Ferroxidase activity
Fe^{2+} through channel → Fe^{3+} in core

H-rich ferritin in heart & brain
average <1000 Fe atoms/molecule

L-ferritin subunit

Serum ferritin = $L_{24}H_0$ (iron-poor)

L-rich ferritin in liver & spleen
average 1500 Fe atoms/molecule

Selective ↑ L-ferritin mRNA with Fe loading

the ferritin mRNA (Figure 8). In iron-depleted states, IRPs bind and prevent translation of the ferritin mRNA. The erythroid form of 5-amino-laevulinic acid (ALA) synthase is also regulated in this manner, linking iron status to porphyrin synthesis. The 3′ UTR of transferrin receptor mRNAs contain 5 potential IREs. In iron-depleted states, IRPs bind to these elements and obscure a degradation signal in the region. The level of transferrin receptor mRNA increases because of its increased stability and there is concomitant increased expression of transferrin receptors (Figure 8).

In iron-replete states, iron binds to the IRPs. IRP-1 in the fully iron-bound form (4Fe in a cluster with 4 sulfur atoms liganded to three cysteines) acquires aconitase activity, catalyzing the conversion of citrate to isocitrate in the Krebs cycle (Figure 7). IRP-1 thus represents a bi-functional protein, cytosolic aconitase action and iron regulation. IRP-2 is degraded when iron-bound (Figure 7). IRPs cannot bind to their cognate IREs when iron-bound, therefore ferritin synthesis proceeds, providing apoferritin for storage of additional intra-cellular iron (Figure 9). Transferrin receptor mRNA is unstable in iron-replete cells. A degradation signal in the 3′ UTR is no longer obscured by IRPs and mRNA levels decrease with subsequent decreases in transferrin receptor expression and receptor-mediated uptake of iron-bound transferrin. The intra-cellular level of iron is thus tightly regulated to permit the uptake of essential quantities of iron and to prevent the toxic effects of higher amounts. IRPs are normal in HLA-linked hemochromatosis.[20]

Ferritin:

Ferritin is the protein that allows the biological storage of iron in an inert form.[17] Each intra-cellular ferritin molecule is a large heteropolymer (~480 kDa) that consists of 24 subunits of L- (light/liver,

Table II: Cellular Proteins Involved in Iron Metabolism

Protein	Chromosome	Function
Transferrin (mwt 80kDa)	3	Iron transport in plasma
Transferrin receptor (180kDa homodimer)	3	Iron transfer to cells
L-ferritin (mwt 19kDa)	11	Ferritin subunit (heart & brain)
H-ferritin (mwt 21kDa)	19	Ferritin subunit (liver & spleen)
Ferritin (mwt ~480kDa)	-	Iron storage (4500 atoms/mol)
Iron regulatory proteins (1= aconitase; 2≠ aconitase)	9 15	Regulate synthesis of ferritin and transferrin receptor

19kDa) and H- (heavy/heart, 21kDa) ferritin (Figure 10). Serum ferritin is a homopolymer of L-ferritin subunits only.[17] The H-ferritin subunits have ferroxidase activity catalyzing the conversion of Fe^{2+}, that accesses the core of the molecule through channels, into Fe^{3+}. The L-ferritin subunit is involved in forming the central iron core from Fe^{3+} and in homopolymers *in vitro* builds iron-cores more slowly.[17] When intracellular levels of iron exceed need, ferritin is synthesized and the additional iron is stored in an inert form. The exact source of circulating serum ferritin is unknown. All tissue sources of ferritin contain at least some H-ferritin subunits, whereas serum ferritin is a homopolymer of L-ferritin subunits. Besides deriving iron from transferrin and non-transferrin bound sources, hepatocytes may additionally take up ferritin by a process of receptor-mediated endocytosis,[21, 22] providing another portal of entry for iron into the liver and potentially contributing to iron overload. The receptor has not been characterized at the molecular level. Whether receptor-mediated uptake of ferritin is important in determining serum ferritin levels is unknown. Overall, the serum level of ferritin reflects total body iron stores. Ferritin is normal (except for levels) in HLA-linked hemochromatosis.

Hemochromatosis Gene

Despite the precise identification and chromosomal localization of each of the cellular proteins involved in iron metabolism, the hemochromatosis gene remained a mystery (Table II). The initial demonstration of recessive inheritance was based on linkage to HLA-A3 on chromosome 6p21.3, reported by Simon *et al.*[3] Before that, there was debate as to whether the inheritance was autosomal dominant with incomplete penetrance and expressivity, perhaps because homozygote-heterozygote matings are not rare.[23] Both HLA-A3 HLA-B7 and HLA-A3 HLA-B14 haplotypes are linked to hemochromatosis.[24] The HLA-A3 HLA-B7 haplotype is common, being found in 10.7% (29/270) Caucasians and 6.1% (3/49) African-Americans in Dallas (P. Stastny, personal communication). The HLA-A3 HLA-B14 haplotype is less frequent (1.5% (4/270) Caucasians in Dallas). The HLA region of the genome is characterized by marked linkage disequilibrium such that informative recombination events are less common than in other areas, making the gene more difficult to locate. With the advent of microsatellite markers, which can be used to identify polymorphisms at many additional sites within the area of interest, the position was refined somewhat, as being probably telomeric to HLA-A3 (Figure 11). By 1993, the gene was localized to a region identified by the anonymous markers D6S265 (centromeric to HLA-A) and D6S105 (telomeric to HLA-A). However, investigators differed in the closeness to HLA-A and the actual distance between the two markers was unknown. In 1995, an "ancestral chromosome", exclusively associated with hemochromatosis, was defined as the haplotype D6S265-1, HLA-A3, D6S105-8[25] and new polymorphic microsatellite markers placed the gene telomeric to D6S105. The gene was finally identified by scientists at the biotechnology company, Mercator Genetics.[4]

The successful approach was that of assembling a set of 50 yeast artificial chromosomes (YACs) and 87 sequence-tagged sites (STSs) that included ~6Mb DNA telomeric from D6S265, the centromeric limit. The genetic distance was <1 centimorgan, suggesting a physical distance of <1 million base

Figure 11: The Hunt for the Hemochromatosis Gene

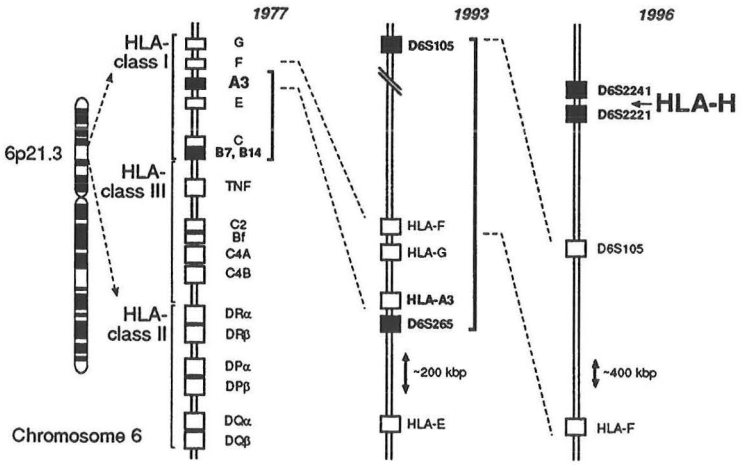


Figure 12: HLA-H Protein
(modelled after MHC Class I)

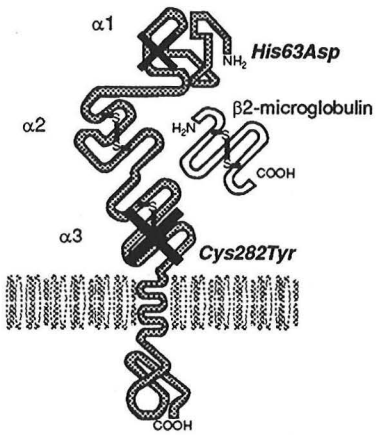


Table III: *HLA-H* — the Hemochromatosis Gene? YES

Source	Cys282Tyr Cys282Tyr	Cys282Tyr His63Asp	Other
U.S.	148/178 (83%)	8/178 (4%)	22/178* (13%)
U.S.	121/193 (82%)	8/147 (5%)	18/147* (12%)
Australia	112/112 (100%)	0/112	0/112
France	59/65 (91%)	3/65 (5%)	3/65* (5%)

*U.S.1: 1=Cys282Tyr/wt, 1 His63Asp/His63Asp, 7 His63Asp/wt, 13=wt/wt;
*U.S.2: 2=Cys282Tyr/wt, 2 His63Asp/His63Asp, 4 His63Asp/wt, 10=wt/wt;
*France: 1=His63Asp/His63Asp, 2=His63Asp/wt

pairs, much smaller than the actual physical distance of >6 million base pairs. This latter discrepancy is the result of the low recombination frequency, since recombination events are used to define the genetic distance. The region containing the gene was further refined to 600kb by the calculation of two statistically independent values, the *P*excess value, a measure of linkage disequilibrium at each marker, and the parameter F, a measure of deviation from Hardy-Weinberg equilibrium. Analysis of this region for recombination events in ancestral chromosomes defined a minimal region of 250kb.

Potential genes within this 250kb region were identified by cDNA selection, exon trapping and complete genomic sequencing. These techniques identified 12 histone genes and 3 novel genes. The novel genes had similarities to the 52kDa Ro/SSA ribonucleoprotein, a sodium phosphate transporter and HLA-A2. All 15 potential genes were sequenced in two patients homozygous for the ancestral chromosome and two controls. This yielded 15 silent polymorphisms (nucleotide change without amino acid change), 2 amino acid changes in histone genes and 1 amino acid change, Cys282Tyr, in the HLA-like gene. As Sherlock Holmes said, "....when you have eliminated the impossible, whatever remains, however improbable, must be the truth", therefore the presumption is that the HLA-like gene encodes the hemochromatosis gene product.

Genetic persistence:

The available evidence strongly suggests that HLA-linked hemochromatosis is due to a single point mutation. The "founder" mutation has persisted at a very high frequency, why? What is the advantage of the heterozygous (or homozygous) state? Speculation about this question usually leads to the assumption of survival advantage provided by protection from iron deficiency. Clearly, women would benefit most from such an advantage. Many women spend much of their reproductive lives on the edge of iron deficiency because of menstrual blood losses and losses to the fetus during pregnancy. Consequently, women are protected from the deleterious effects of the gene until later in life. In addition, men often do not develop symptoms or signs of iron overload until the fifth decade, well beyond the life expectation of early man. We can only continue to speculate at this time. As we learn more about this common disease, perhaps we'll come closer to the truth.

HLA-H — THE HEMOCHROMATOSIS GENE PRODUCT

The protein encoded by the HLA-A-like molecule is predicted to resemble HLA class I molecules with a potential peptide-binding domain, an immunoglobulin-like domain, a single transmembrane region and a short cytoplasmic tail. The authors used the name *HLA-H* for their gene, HLA-H for its protein. This terminology awaits official confirmation, since the term *HLA-H* has been used for a pseudogene (stop codon in exon 4) within the MHC class I region.[26] The term *HFE* has been previously used for hemochromatosis.[27] All the ancestral chromosomes contained a G → A transition [G845A], a missense mutation that changes the amino acid at position 282 from a cysteine to a tyrosine [Cys282Tyr]. Cys282 is predicted to form part of the intra-molecular disulfide bridge in the α3-like domain of the molecule (Figure 12). 148 of 178 (83%) clinical

hemochromatosis patients were homozygous for this mutation. An additional 9 patients were heterozygotes.

A second missense mutation, His63Asp, was identified exclusively in chromosomes NOT containing Cys282Tyr. This mutation, while relatively common in control subjects (51 of 308 chromosomes, 17%), was found in 89% of Cys282Tyr heterozygotes (8/9). In 21 patients, neither chromosome carried the Cys282Tyr mutation. Further analysis revealed only His63Asp in 9 (21%, not different from controls). No other mutations were detected. The 21 patients may represent a different disease since evidence of familial iron overload was not a pre-requisite for the collection of clinical sample. When 5 of these 21 patients were re-examined, 1 had a history of self-administering iron supplements for >30 years.[28] None of the remaining 4 patients had a family history of iron overload. There mean age was younger (36 ± 8 years) than those homozygous for Cys282Tyr (48 ± 2 years) yet their hepatic iron indices were higher at 9.8 μmol/g/yr compared with 4.7 μmol/g/yr for the Cys282Tyr homozygotes (see Liver biopsy section in Diagnosis of Hemochromatosis below for explanation).[28] Additional studies are needed to ascertain the cause of their iron overload.

Confirmation of mutation(s):

Because there was no easy explanation for the clinical syndrome associated with the putative disease gene product, the initial concern was that it may not represent the true hemochromatosis gene. However, this fear was soon dispelled with the rapid confirmation of the mutation(s) by three independent groups, including one from Europe and one from Australia (Table III).[29-31] The finding of 100% concordance with Cys282Tyr in the carefully selected families analyzed by Jazwinska *et al*, suggests that this may be the sole mutation in "pure" HLA-linked hemochromatosis. Whether the compound heterozygous state, Cys282Tyr/His63Asp, can lead to iron overload in the absence of another confounding variable is unclear. The penetrance of clinically diagnosed hemochromatosis from this combination is calculated to be low.[29]

The function of HLA-H in iron metabolism is unknown. The mRNA is detected in most tissues except brain, with highest expression in the liver and small intestine.[4] The normal molecule is predicted to be 343 amino acids and expressed on the cell surface, a localization for which Cys282 is critical.[32] Thus, a similar mutant protein expressed from an *H-2L*^{d} gene (murine equivalent of an HLA class I molecule) failed to appear on the surface. It was found in large quantities in the cytoplasm, in a glycosylated form and associated, at least in part, with β2-microglobulin.[32] Intra-cellular localization studies, using antibodies directed to a C-terminal peptide, demonstrate a plasma membrane distribution in non-polarized cells such as esophageal epithelial cells and leukocytes.[33] Basolateral membrane distribution was observed in polarized epithelial cells from the gastric antrum, colon and gall bladder. In the liver, the expression was basolateral in bile ductular epithelium and sinusoidal lining cells also expressed the protein. The small intestine demonstrated primarily intracellular and perinuclear localization in crypt cells, with highest expression in the duodenum. The correlation between immunohistochemical localization and function remains unknown. Proposed functions for HLA-H include binding an iron-related ligand, signal transduction regulating iron transfer and immune system association.[4]

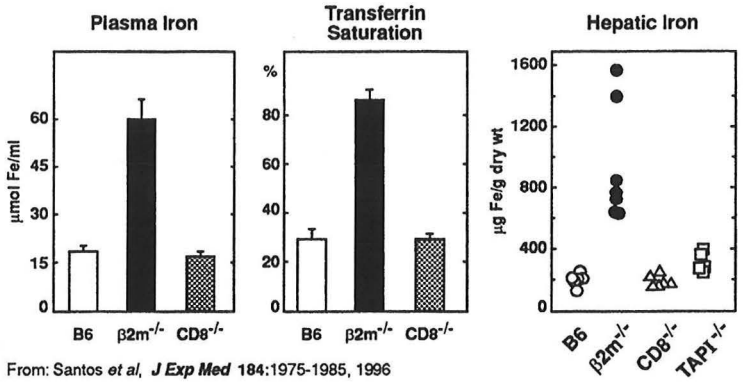
Animal Models

Progress in the understanding of the pathogenesis of genetic diseases can be greatly aided by the availability of appropriate animal models. Many investigators have tried, mostly unsuccessfully, to develop a model system that mimics hemochromatosis.[34] There is iron accumulation in the livers of some birds with associated fibrosis/cirrhosis of the liver however increased dietary intake of iron, rather than increased absorption, may explain these findings.[34] Iron also accumulates in the livers of hypotransferrinemic mice,[35] a model for human atransferrinemia. Feeding iron in various forms results in iron overload in rodents but the pattern is not similar enough to clinical hemochromatosis to provide a model.[34]

β2-microglobulin deficient mice:

In 1994, de Sousa and colleagues reported that β2-microglobulin deficient mice develop iron overload.[36] In these mice, cell surface expression of all HLA class I molecules is severely decreased, including, presumably, the murine equivalent of HLA-H. This observation was confirmed by Rothenberg and Voland in 1996.[37] They extended the findings, documenting progressive iron deposition in the liver of β2-microglobulin deficient mice which increased with increased dietary iron.

Figure 13: Increased Iron Levels in 12 month old β2-microglobulin Deficient Mice



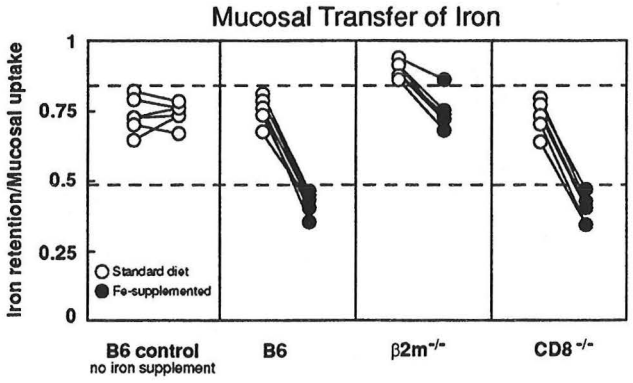
From: Santos *et al*, ***J Exp Med*** 184:1975-1985, 1996

Spontaneous liver tumors[37] are also observed in these mice.

More recently Santos and co-workers have carried out a series of elegant experiments in *β2*-microglobulin deficient mice (Figures 13 and 14). They demonstrated a 4-fold increase in serum iron concentrations, increased transferrin saturation (>80%) and increased hepatic iron when compared with normal control mice of the same strain (Figure 13). Of great importance, mice that lacked $CD8^+$ cells and mice that lacked the endoplasmic reticulum transporter for class I-associating peptides ($TAP^{-/-}$) were similar to control mice. Thus, neither the lack of $CD8^+$ cells in the *β2*-microglobulin deficient mice nor the lack of a peptide presented on the cell surface by a class I-like molecule can mimic the findings. These observations suggest that the HLA-H molecule itself, rather than a peptide bound to the molecule, is critical in regulating iron transfer. A non-peptide could conceivably bind to the peptide-binding region. For example, lipid moieties such as isopentenyl pyrophosphate and farnesyl pyrophosphate are capable of binding to antigen presenting cells and being specifically recognized by $\gamma\delta$T cells.[38]

In other experiments, these investigators measured intestinal iron uptake and transfer in the mice (Figure 14).[39] They observed increased mucosal transfer of iron, regardless of iron stores, thereby confirming the similarity to HLA-linked hemochromatosis. The role of reticuloendothelial cells in determining the pattern of iron distribution was examined by transplanting normal hematopoietic donor cells into lethally irradiated, *β2*-microglobulin deficient, recipient mice. In these animals, the site of iron storage altered from hepatocytes (parenchymal cells) to Kupffer cells (reticuloendothelial cells of donor origin).[39] Thus, this model faithfully reflects many of the abnor-

Figure 14: Increased Iron Transfer in 2 month old β2-microglobulin Deficient Mice



From: Santos *et al*, ***J Exp Med*** 184:1975-1985, 1996

Figure 15A: Hepatic Metabolism of Dietary Iron

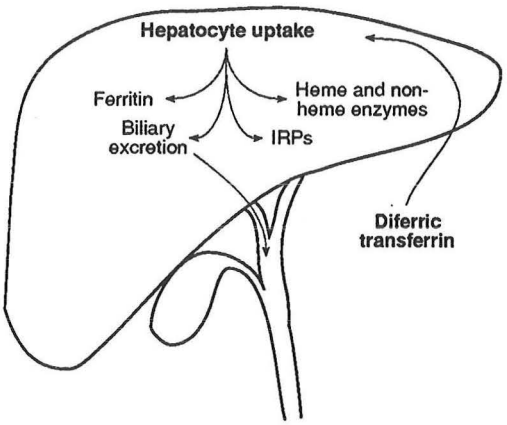
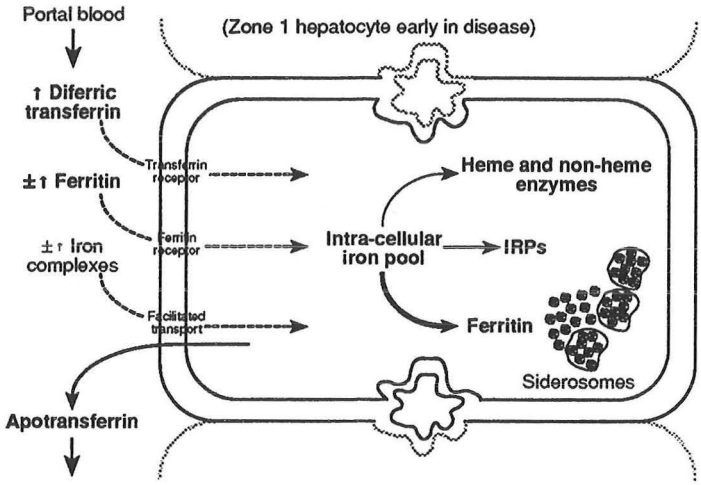


Figure 15B: Hepatocyte Metabolism of Iron — Early



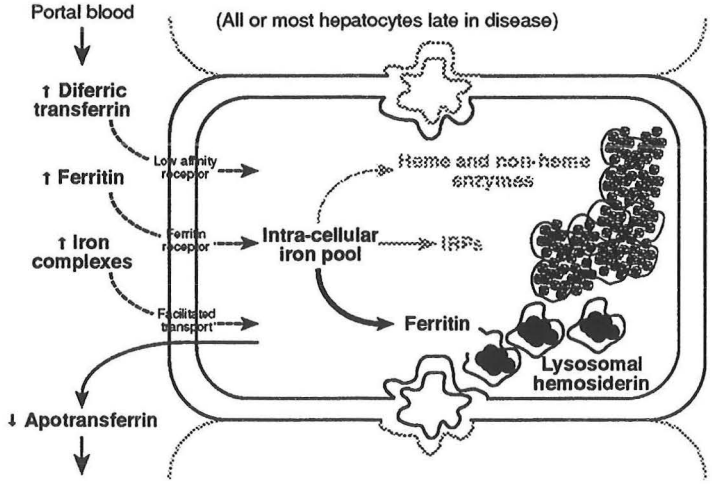
malities observed in HLA-linked hemochromatosis: 1) increased and inappropriate mucosal transfer of iron; 2) elevated serum iron, transferrin saturation and hepatic iron content; 3) parenchymal distribution of liver iron with paucity of reticuloendothelial cell deposition. Taken together with the identification of the Cys282Tyr mutation in the HLA-H gene, it would appear that one of the mysteries of HLA-linked hemochromatosis, that of which gene is responsible, has been solved.

Pathogenesis of Hemochromatosis

In hemochromatosis, iron enters the parenchymal cells of the liver from transferrin, non-transferrin bound iron in complexes and ferritin in the portal blood (Figure 15). Both high affinity transferrin receptors and a low affinity, high capacity pathway may contribute to the increased deposition of transferrin-bound iron in the hepatocytes (Figure 15). This possibility is suggested by the finding that the expression of the high affinity transferrin receptor is appropriately down-regulated in hemochromatotic livers. Such down-regulation should serve as an effective mechanism to prevent hepatocyte uptake of additional iron once cells are replete. Increased intrahepatic iron also decreases transferrin levels, resulting in the characteristically low total iron-binding capacity in advanced hemochromatosis. If the continued increase in hepatic iron cannot be explained by uptake of non-transferrin bound iron and ferritin, then a low affinity, high capacity, unregulated pathway for acquisition of transferrin-bound iron by hepatocytes could account for the observations.[15] Because the iron enters the liver from the portal system, periportal hepatocytes have a higher iron content than pericentral cells.

Excess intra-cellular iron is stored in ferritin in membrane-bound siderosomes, considered to be precursors of hemosiderin-laden lysosomes

Figure 15C: Hepatocyte Metabolism of Iron — Late



(Figure 15). These deposits can be readily visualized using Perl's Prussian blue stain. The zonal nature of iron content (periportal > pericentral) can also be observed. When undiagnosed and untreated, the increase in total body iron continues inexorably. Finally, the liver and pancreas contain 50- to 100-fold increases in iron, averaging 20g in the liver.[40] The heart contains a 10- to 15-fold increase in iron content, whereas spleen, kidneys and skin demonstrate approximately 5-fold increases.[40] In contrast, bone marrow stores can be normal or merely modestly elevated[41, 42] until late in the course of HLA-linked hemochromatosis. This is likely explained by 2 separate phenomena: 1) dietary origin of excess iron and 2) abnormally high release of iron from reticuloendothelial cells.[43] In 2° iron overload, in contrast, bone marrow reticuloendothelial cells can store 10g of iron derived from transfused blood (40 units).

Iron-mediated tissue injury:

The accumulation of excess iron leads to tissue damage. The mechanism of injury is thought to be that of lipid peroxidation resulting from the intra-cellular generation of reactive oxygen species.[44] The decomposition of structural lipids, for example, polyunsaturated fatty acids in membrane phospholipids of organelles, is one possible mechanism to account for damage and fragility of lysosomes, mitochondria and other organelles, leading to cell injury/cell death. In addition, there is DNA damage, demonstrated by increased strand breaks in hepatic DNA in animal models. Bulky DNA damage has been detected in liver tissue from hemochromatosis patients.[45] DNA damage is likely to be related to the >100-fold increased risk of developing hepatocellular carcinoma in these patients. Hepatic fibrogenesis is observed after the liver exceeds a certain critical threshold of iron content. In early stages, there is increased iron but no fibrosis (Case #2). When the iron content surpasses ~22,000-33,000 μg/g dry wt (~400-600 μmol/g), however, fibrogenesis ensues.[46, 47] This is mediated by activation and proliferation of the Ito cells (stellate cells, lipocytes, fat-storing cells) in hepatic sinusoids, probably a direct result of lipid peroxidation damage. Other factors, however, such as alcohol consumption and coincident viral liver disease can greatly lower the threshold for fibrosis, with development of cirrhosis before the iron content reaches 22,000 μg/g (Case #1).[48, 49]

Hepatic iron content increases with age initially,[46, 50] however, saturation of hepatocytes is approached eventually.[51, 52] This is in contrast to serum ferritin levels which continue to rise and therefore reflect total body iron stores (Figure 16). In Italian patients, the saturation level was an iron content of ~20,000μg/g.[51] However, the content in individuals can exceed 55,000μg/g dry wt (>1,000μmol/g).[46, 52, 53] Some caution is needed in interpreting the values obtained for hepatic iron determination. Thus, measurements of the iron content in multiple samples from explanted livers demonstrated intra-hepatic variability.[54] Potential sources of variation include the amount of fibrous tissue in the sample and presence within the parenchyma of iron-free foci. Such occurrences are predicted to lower the hepatic iron content falsely. These artifacts can be circumvented by excising tissue embedded in paraffin blocks, thereby avoiding non-representative areas.[55] Measurements of iron made on paraffin-embedded material are comparable to those on freshly analyzed tissue.[56] A more important source of interpretation error

Figure 16: Saturability of Hepatic Iron Stores and Threshold for Iron-mediated Fibrosis

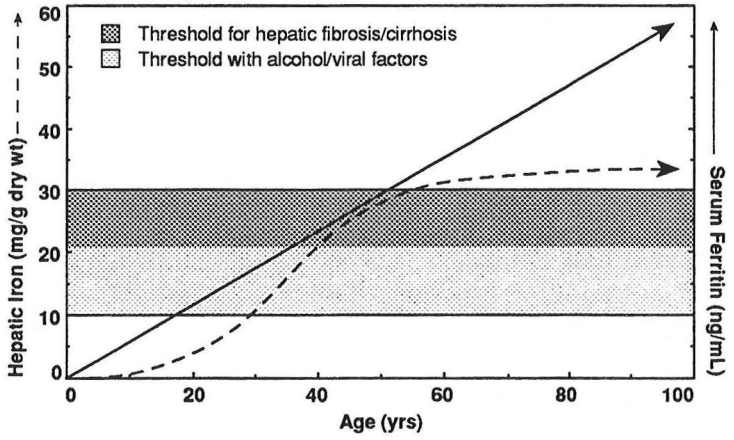
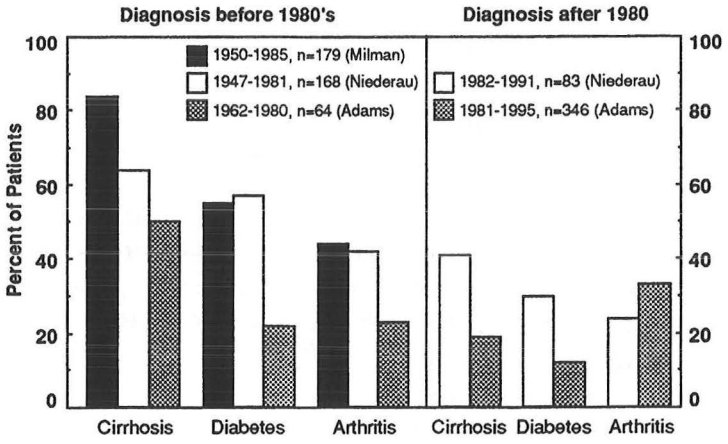


Figure 17: Changing Presentation of Hemochromatosis



is that of overlooking the contribution of alcohol consumption to the distribution of iron within tissues. LeSage and colleagues found that patients could have different hepatic iron content despite equal iron overload as estimated by iron removed at phlebotomy.[53] In the group of patients that consumed excessive quantities of alcohol, the hepatic iron content was 60% of that in non-drinkers (17,344 μg/g compared with 28,553 μg/g) whereas mobilizable iron was identical (19.6g compared with 19.5g). Alcohol apparently was associated with the redistribution of iron away from the liver.

Other organs become damaged by iron overload when iron continues to accumulate. Iron deposition in the pancreas, heart, joints, endocrine glands and skin leads to cellular injury and degeneration with resultant fibrosis. The heart appears to be particularly vulnerable if the iron accumulation is rapid, as evidenced by early presentation (<30 years of age) with symptomatic disease. Any of these tissues may be the origin of symptoms or signs that eventually lead to the diagnosis of HLA-linked hemochromatosis.

Clinical Presentations

HLA-linked hemochromatosis may be diagnosed in patients presenting with hepatic, cardiac, endocrine, rheumatologic or dermatologic symptoms or signs or patients may be asymptomatic at the time of diagnosis. The main determinants of the mode of presentation include: 1) the process of case ascertainment, being either symptoms/signs in the proband or as a family member undergoing evaluation[47] or follow-up of routine screening tests (Case #2); 2) the presence or absence of other factors causing tissue/organ damage such as alcohol consumption (Case #1), viral liver disease, other risk factors for diabetes and cardiac disease; and 3) genotype, in that the ancestral genotype has been associated with more severe disease manifestations.[57, 58] Differences in other genes or environmental factors regulating iron uptake, transfer, distribution or removal must also necessarily affect the magnitude of the pertinent findings.

Because HLA-linked hemochromatosis is generally being diagnosed at an earlier stage than previously, the proportion of patients with cirrhosis and diabetes at initial presentation has decreased substantially (Figure 17), from 84% and 55% respectively (Milman *et al*, diagnosed 1950-1985)[59] to 7% and 9% (Adams *et al*, family members diagnosed 1981-1995).[47] In contrast, there has been little change in the percentage with arthropathy, 44% and 33% respectively. Presentation with common but non-specific complaints such as weakness and/or lethargy (82%) and abdominal pain (56%)[51] may provide diagnostic difficulties and delay establishment of the etiology.

Liver disease:

Non-specific complaints such as fatigue, weakness and lethargy may be the only overt indication of disease until complications of cirrhosis intervene. Hepatomegaly is very common (>80%) in the large case series of Finch and Finch,[40] Milman[59] and Niederau *et al*[51] and may be the source of the abdominal pain present in up to 56% of subjects.[51] Jaundice and clinical signs of portal hypertension are not observed until very late in the course. Even in the large group of patients reported by Finch and Finch in 1955, before the

introduction of routine phlebotomy treatment, only 25% had abnormal bilirubin level. Standard laboratory investigations are completely normal (Case #1) or only mildly increased in the absence of co-factors that also cause liver disease. In two case series of 100 or more patients, AST/ALT were normal in 35%/31%[60] and 80%/47%.[61] The higher incidence of abnormal ALT (53% and 69%) contributes to the finding of subjects with iron overload/HLA-linked hemochromatosis when the cause of an abnormal ALT discovered at blood donation is determined (1 in 100 donors had hemochromatosis).[62]

Unfortunately, the failure to pursue the cause of serum aminotransferase elevation is very common.[63] Meyer *et al* reviewed the records of 100 patients selected for persistent low grade (<2-fold) increases in both AST and AP.[63] They found that hemochromatosis was not excluded in 90% of patients. In 30 patients, there was no mention in the chart that abnormalities were present; in 39 patients, abnormalities were ascribed to other conditions without considering the possibility of co-existent iron overload and in 21, the condition of the patient was so poor that further evaluation was not indicated.[63] In Italy, co-existence of iron overload with viral liver disease is rather frequently observed (hepatitis B, 5% and hepatitis C, 21%)[48] emphasizing the need to consider the diagnosis in all patients. Some patients with hepatitis C have moderate iron overload (not hemochromatosis). When they are treated with phlebotomy, ALT levels that were previously abnormal can improve or normalize.[64, 65] This observation suggests that iron can contribute to hepatic damage even at moderate levels of accumulation if there is co-existing viral liver disease. Additional evidence of the frequency with which iron overload can co-exist with other causes of liver disease is obtained from the reports of transplantation for complications of liver disease. Hemochromatosis was not suspected before transplantation in 7 of 9 patients in one series[66] and 13 of 37 in another.[67] Common etiologic factors contributing to progressive liver disease in these subjects were alcohol and hepatitis C.

Radiologic investigations — Radiologic investigations may provide the first indication of iron overload. Both computed tomography (CT) and magnetic resonance (MR) imaging studies have distinctive characteristics. On CT, there is increased density over the iron-loaded liver when compared with the spleen or other organs/tissues.[68] However, CT changes are insensitive, particularly if fatty infiltration is present such that the averaged signal returns towards normal.[69] MR imaging demonstrates a reduction in signal intensity related to a decrease in T2 relaxation time.[69] This approach does not permit accurate evaluation of iron stores <5 times normal, however.[69] Alternative measurements of liver-to-tissue signal intensity ratios detect less iron overload and correlate with tissue iron content[70, 71] but may be less accurate at higher iron content. Neither CT nor MR is reliable enough to replace liver biopsy and assaying iron directly.

Complications of cirrhosis — With continued iron accumulation and development of cirrhosis, complications of portal hypertension can supervene, although much less commonly than in Laennec's cirrhosis.[40] In the cases reviewed by Finch and Finch, ~30% died from hepatic failure or gastrointestinal hemorrhage.[40] Niederau *et al* found that only 13 of 251 patients presented with the combination of ascites, splenomegaly, esophageal varices and impaired liver function, 9 died before iron depletion could be completed.[51] Individual findings compatible with decompensation were more common, peripheral edema in 23%, jaundice in 19% and ascites in 13%. The difference between rates of peripheral edema and ascites may represent a contribution of cardiac failure.

Malignancy — Hepatocellular carcinoma occurs at a higher rate in HLA-linked hemochromatosis than in any other adult liver disease. There is a >100-fold increase in risk of hepatocellular carcinoma compared with the general population and the incidence of cholangiocarcinoma is also increased. The risk persists after successful iron depletion, accounting for 15 of 35 deaths (43%) following complete removal of iron in the patients followed by Niederau *et al*.[51] Indeed, the relative risk of succumbing to hepatocellular carcinoma increases with successful phlebotomy. Only 4 of 34 deaths (12%) that occurred before iron depletion in the Niederau *et al* series were attributed to hepatocellular carcinoma[51] and Finch and Finch in their 1955 series reported an overall incidence of 14%.[40] Although strongly linked to an underlying cirrhosis, hepatocellular carcinoma has also been reported rarely after "reversal" of cirrhosis by venesection,[72, 73] in patients with fibrosis[74] and in one patient with an otherwise normal liver architecture at hepatic resection.[75]

The observation of spontaneous liver tumors in β2-microglobulin deficient mice provides one animal model for understanding the pathogenesis of malignancy.[37] Another animal model may be that of hepatocellular carcinoma in the LEC rat.[76] This animal model of Wilson's disease (see reference 77 for review) differs from the human equivalent in that hepatocellular carcinoma occurs in >90% of animals that survive the early fulminant hepatic failure.[77] Recently, Kato and coworkers have demonstrated that hepatic iron deprivation prevents the spontaneous development of both fulminant hepatitis and hepatocellular carcinoma in these animals.[76] Iron accumulation may act synergistically with copper to produce the changes that lead to malignancy in this model.

Endocrine disease:

Diabetes mellitus is present in increasing numbers of patients as the level of iron overload increases. Finch and Finch reported that 82% of the patients they reviewed in 1955 developed diabetes.[40] In contrast, diabetes was present in only 9% of 133 patients discovered by screening family members of Canadian and French probands.[47] The median year of diagnosis for all patients (probands and discovered cases) was recent, 1987 for 167 Canadian patients and 1992 for 243 French patients.[47] Both deposition of iron in the pancreas and insulin resistance related to liver disease are likely contributors to the development of diabetes mellitus. Both insulin-dependent and non-insulin-dependent disease are encountered.

Hypogonadotrophic hypogonadism is also relatively common.[78] Impotence was reported by 81 of 224 patients (36%) in one recent series[51] and 40 of 99 patients in another.[47] Accompanying findings were loss of body hair in 16% (39/251)[51] and amenorrhea in 4 of 27 women (15%).[51] Both impotence and loss of body hair were more common in those with cirrhosis (43% and 23% respectively) than in those without cirrhosis (27% and 6%).[51] Although most instances of hypogonadism in iron overload are attributed to or demonstrated to be of pituitary origin,[78, 79] occasional patients with hypothalamic or testicular origin are reported.[79-81] Less common endocrine manifestations of iron overload include hypothyroidism and hypoparathyroidism.[82, 83]

Cardiac disease:

In iron overload, a dilated cardiomyopathy is the commonest finding.[84] Rarely, a restrictive cardiomyopathy can occur.[85] Iron is deposited in cardiac myocytes, leading to the suggestion that the disease is one of a disturbance 2° to storage rather than infiltration.[86] Systolic function is generally preserved until a critical concentration of iron is obtained.[84] However, exogenous stresses may result in overt cardiac failure when routine evaluation does not demonstrate evidence of significant dysfunction.[66] Thus, 3 patients with (undiagnosed) hemochromatosis undergoing liver transplantation developed congestive heart failure and 4 patients developed arrhythmias postoperatively.[66] Once the critical iron content is reached, cardiac failure with rapid deterioration can occur over a short period of time. Before 1955, cardiac failure was the commonest cause of death.[40]

The patients with a higher likelihood of developing cardiac failure are those with more rapid accumulation of iron and consequent presentation with symptomatic disease in childhood[87] or early adulthood[87-90] (reviewed in reference 91). This is in sharp contrast to the mean age at presentation of 49 yrs in probands and 48 years in family members discovered on screening by Adams and collaborators.[47] Of 16 children between the ages of 4 and 19 years who presented with symptoms related to iron overload, 11 died within 2 years of diagnosis, most from congestive heart failure. The mechanisms responsible for the rapid development of iron overload have not been identified. Either genetic or environmental factors might be responsible for these effects.

The specific diagnosis of iron deposition in the heart can be difficult because of the patchy nature of the disease, the risks of myocardial biopsy and the relative insensitivity of non-invasive modalities such as MR imaging. An indirect diagnosis, of hepatic involvement with HLA-linked hemochromatosis and consistent cardiac features, can usually suffice. More controversial is the question of whether body iron stores have a general association with the risk of ischemic heart disease. Salonen *et al* have reported a 2.2-fold increased risk of myocardial infarction in Finnish men with increased serum ferritin levels (≥200ng/ml).[6] It is conceivable that lipid peroxidation may be catalyzed by iron even at relatively low levels of total body iron, when the vast majority of the iron should be bound to various protein moieties and therefore inert. Alternatively, the increased ferritin may reflect inflammation rather than body iron stores. This latter possibility is supported by the failure to demonstrate any relationship between transferrin

saturation and ischemic heart disease in the United States.[92]

Arthropathy and bone disease:

Symptoms related to bone or joint involvement have increased in relative frequency recently (see Figure 17). Whereas Finch and Finch did not quantify the occurrence in their cases,[40] both the large German and Danish case series reported an incidence of 44%.[51, 59] The incidence was lower (32%) in the French and Canadian probands followed by Adams and collaborators, and 21% in patients identified by screening family members.[47] After pigmentation (38%), however, arthritis was the most common finding.[47] The usual manifestations include metacarpophalangeal and proximal interphalangeal arthritis, large joints are affected less often.[82, 93, 94] There may be progressive chondrocalcinosis and calcium pyrophosphate crystal deposition. Osteonecrosis of the hip is also observed (Case #1). Unlike most other manifestations of iron overload, the arthritis does not improve with phlebotomy.[93] Osteopenic bone disease may also complicate HLA-linked hemochromatosis.[95] The decrease in bone density may be especially significant when hypogonadism is present.[95] Cirrhosis is also associated with an increased incidence of osteoporosis, this appears to be a less important factor.

Dermatologic disease:

"Bronze diabetes" was the original name for the triad of cirrhosis, diabetes mellitus and skin pigmentation. The "bronze" appearance arises from increased melanin in the epidermis, the direct result of iron deposition in the skin and melanocyte stimulation.[96] A slate-grey pigmentation ensues from the iron itself, deposited in the epidermis and sweat glands. Although pigmentation is currently the commonest manifestation of HLA-linked hemochromatosis (38% of 410 French and Canadian patients diagnosed between 1962 and 1995),[47] there has been a substantial decrease from the 72% rate in 251 German patients diagnosed between 1947 and 1991[51] and the 90% incidence in the series of Finch and Finch from prior to 1955.[40] It would appear that the variable incidence is related to the magnitude of body iron stores at the time of diagnosis.

Porphyria cutanea tarda (PCT) has a unique association with HLA-linked hemochromatosis. The disorder is one of decreased activity of uroporphyrinogen decarboxylase in the liver with a subsequent increase in uroporphyrinogen that is excreted in urine (reviewed in reference 97). In the sporadic form of PCT, the enzyme deficiency is restricted to the liver and hepatic siderosis and iron overload are common. Iron is required for the deactivation of uroporphyrinogen decarboxylase and depletion of iron stores is beneficial, enzyme activity returns to normal and the skin disease is ameliorated. The association of sporadic PCT with alcoholism and viral hepatitis suggests that these non-genetic conditions may be responsible for the iron overload observed in many patients. However, 18 of 41 (44%) Welsh patients with PCT had at least one mutated *HLA-H* gene.[98] Seven (17%) patients were homozygous and 11 heterozygous for Cys282Tyr. This unexpected finding suggests that a subset of PCT patients may have iron overload on a genetic basis. Findings consistent with this observation (without mutation analysis) were reported from Italy.[99] Thus, 58 of 94 (62%) PCT patients had iron overload and those with iron overload had a higher frequency of the hemochromatosis-associated HLA-A3 antigen[99] suggesting that the HLA-linked hemochromatosis gene was mechanistically responsible for the iron overload.

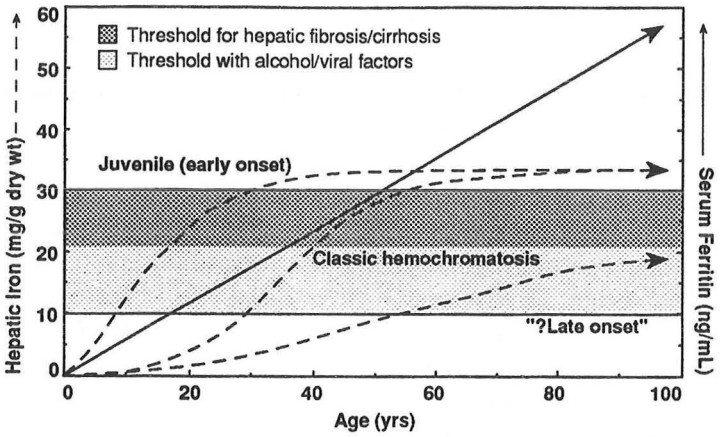
Other clinical manifestations:

Iron is necessary for the growth of all bacteria except some strains of *Bacillus* and *Lactobacillus*. Patients with excess iron are at increased risk of some uncommon infections including *Yersinia enterocolitica* and *Vibrio vulnificans*.[100-103] They may also be at risk for extra-hepatic malignancy.[104, 105] However, this was not confirmed in the most recent review of outcome in 251 German patients,[51] perhaps because of their successful phlebotomy program. As with the association between body iron stores and ischemic heart disease, the purported increased risk of cancer in men with high body iron stores[7] has not been confirmed in other studies.[106] The available evidence would indicate that the association is unproven.

Unusual clinical presentations:

"Juvenile" hemochromatosis presents a distinct entity, as noted above (see Cardiac disease in Clinical Presentations). Pediatric cases lack the marked male predominance of adult hemochromatosis and have a disproportionate preponderance of cardiac presentations. Families with more than one child developing symptomatic iron overload under the age of 10 years[107] suggest the possibility of additional interacting factors contributing to early disease. For example, there may be a synergistic

Figure 18: Saturability of Hepatic Iron Stores and Threshold for Iron-mediated Fibrosis



effect of a mutation or polymorphism in a different gene product involved in mucosal iron uptake or transfer. A similar environment for each family member also raises the possibility of an exogenous factor interacting with a genetic component. This would be analogous to the genetic and environmental combination encountered in the iron overload of sub-Saharan Africa.[108] The discrepancy between iron stores in some HLA-identical siblings[109, 110] also suggests the possibility of genetic or environmental interactions to determine the final level of iron stores. In general, there is concordance of iron storage in HLA-identical siblings but a wide range of values between different sibling pairs[111] supporting either an environmental factor or a genetic factor not linked to the HLA region.

"Late onset" hemochromatosis may also occur. Adams reported that 8 patients who were homozygous for the Cys282Tyr mutation had both normal transferrin saturation and hepatic iron content with no obvious explanation. Failure of phenotypic presentation, like early onset disease, may be related to other genetic or environmental factors that regulate iron absorption.

Diagnosis of Hemochromatosis

Approaches to the diagnosis of HLA-linked hemochromatosis include determinations of iron biochemistry in serum, radiologic investigations (see Liver disease section in Clinical Presentations), biopsy examination and measurements, phlebotomy quantitation of iron stores and now genotyping (previously HLA studies in family members). Each of these approaches has limitations or caveats.

Iron biochemistry:

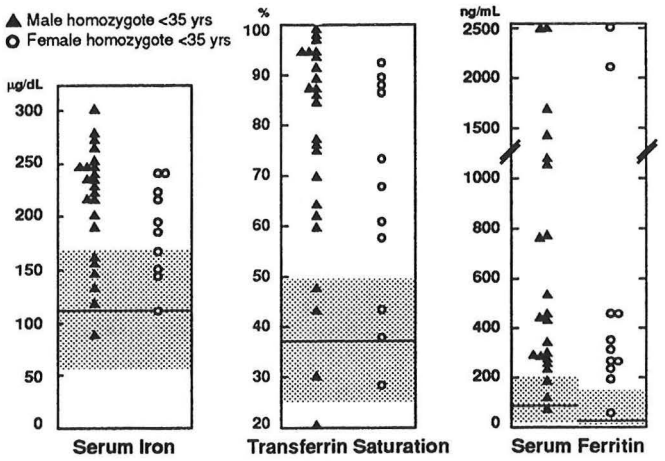
Comparison of measurements of serum iron, transferrin saturation (TS) and serum ferritin have demonstrated that the TS is the most sensitive single test to identify iron overload in HLA-linked

Table IV: Diagnosis of Hemochromatosis

Assay	Normals	Classic Cases	Heterozygotes
Transferrin saturation	4-58%*	>62%	7-85%
Serum ferritin (ng/mL)	26-157* ♂ 19-66* ♀ (95% confidence interval)	↑↑↑	43-160 ♂ 22-98 ♀ (95% confidence interval)
Hepatic iron (μg/g)	<2,000	↑↑↑	mean 1,276 (nl TS) mean 1,776 (↑ TS)
Hepatic iron index	<2	>2	
Iron removed (g)	<5 g	>5 g	

*From: Bulaj *et al*, ***N Eng J Med*** **335**:1799-1805, 1996

Figure 19: Biochemical Detection of Homozygosity



From: Bassett *et al, Gastroenterology* 87:628-633, 1984

hemochromatosis.[112] A level >62% was an effective cut-off to diagnose homozygous HLA-linked hemochromatosis and distinguish between heterozygotes and homozygotes. This cut-off may be too stringent for the diagnosis of homozygosity in younger women with physiologic blood loss through menstruation and pregnancy. In a recent study, Bulaj *et al* demonstrated that TS in 321 normal control subjects (HLA-disparate family members and spouses of hemochromatosis patients) was ≤58%, with mean values of 29-30% in men and 25-26% in women (Table IV).[113] Some male heterozygotes (22 of 505) had initial TS determinations of >62%, on repeat with fasting they were ≤62% in 13 of 13. Two of 553 female heterozygotes had fasting TS levels of 56% and 58%; in another 11 female heterozygotes, non-fasting TS levels that were >50% decreased to <50% when repeated with fasting. This study thus confirms the utility of the 62% cut-off for distinguishing homozygous from heterozygous hemochromatosis and from normals. The results also indicate that screening tests employing transferrin saturation will likely have fewer false positive results if fasting determinations are utilized.

Ferritin — Serum ferritin measurements may be as sensitive as transferrin saturation in the detection of early hemochromatosis (Figure 19 and Table V)[114] and in family studies.[115] More recently, however, Bulaj *et al* observed that serum ferritin levels were higher in heterozygotes than in normal control subjects, with levels in 20% of male heterozygotes exceeding the 95th percentile value of age-matched controls; ferritin levels increased with advancing age in both groups.[113] In addition, 8% of female heterozygotes had ferritin values that exceeded the 95th percentile value for the age-matched female controls. Thus, it would appear that there would be more false positives using serum ferritin to detect homozygous HLA-linked hemochromatosis than with TS. False negative

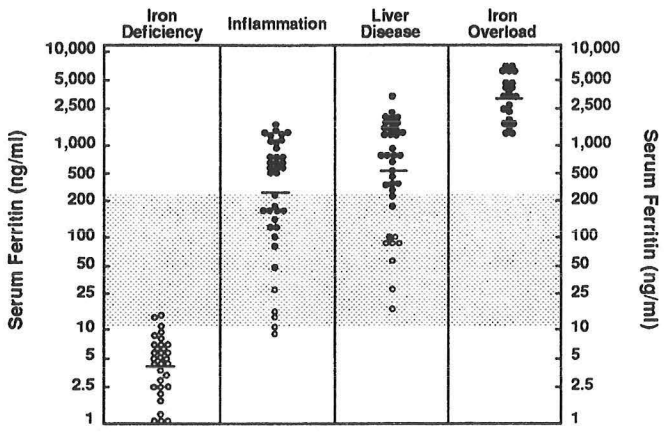
Table V: Detection of Early Hemochromatosis

Assay*	Sensitivity	Specificity	Predictive Accuracy	
			Positive	Negative
Transferrin saturation TS	0.82	0.88	0.74	0.93
Serum ferritin SF	0.85	0.95	0.88	0.94
Serum iron	0.68	0.83	0.61	0.87
TS + SF	0.94	0.86	0.73	0.97

* TS >50%; SF >200µg/ml; serum iron >166µg/dl

From: Bassett *et al, Gastroenterology* 87:628-633, 1984

Figure 20: Serum Ferritin in Disease States



From: Lipschitz, Cook & Finch, *N Eng J Med* 290:1213-1216, 1974

(normal) serum ferritin values are occasionally observed in patients with early disease.[114] In a family study, subjects with putative pre-cirrhotic hemochromatosis but elevated iron stores (5-10g removable iron) had normal serum ferritin values.[116] However, the inheritance pattern of iron overload was considered to be autosomal dominant with variable expressivity, different from that observed in authentic HLA-linked hemochromatosis (except in the situation of homozygote-heterozygote mating). Some doubt exists as to the exact diagnostic entity in this study.

Since ferritin levels are elevated with inflammation and liver disease[117] (Figure 20), an additional element of non-specificity may complicate its use in determining which patients with liver disease have HLA-linked hemochromatosis. The combination of serum ferritin and transferrin saturation was superior to either alone in detecting early HLA-linked hemochromatosis, with a 94% sensitivity, 86% specificity and a negative predictive accuracy of 97%.[114] The positive predictive accuracy was less (73%).[114] The combination of transferrin saturation >62% and elevated serum ferritin is not diagnostic for iron overload nor for HLA-linked hemochromatosis, however, it provides a useful method of non-invasive assessment of iron stores and identifies persons needing additional evaluation.

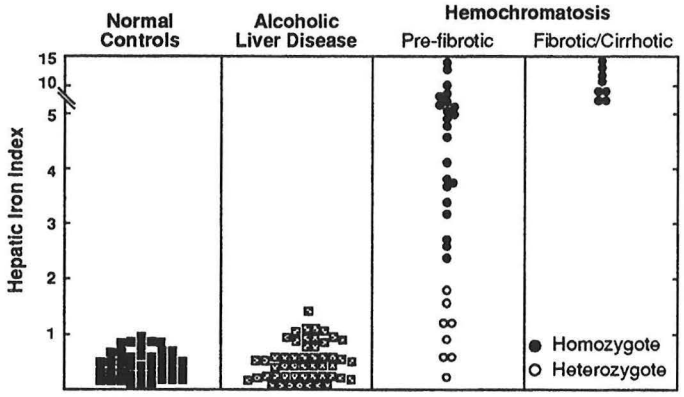
Hyperferritinemia — Isolated hyperferritinemia has been described in combination with congenital cataract.[18, 19, 118, 119] The subjects had normal serum iron and transferrin saturation yet increased serum ferritin levels (>1,000ng/ml). Phlebotomy results in iron deficiency without altering the raised ferritin. The molecular basis of the increase in serum (L-) ferritin has now been explained. They have a point mutation in the loop section of the stem-loop IRE in the 5′ UTR of L-ferritin.[18, 19, 119] Consequently, the L-ferritin mRNA is translated regardless of iron stores. This disorder is readily separated from most iron overload syndromes because the serum iron and transferrin saturation are normal and iron stores are normal or diminished. Hyperferritinemia with iron overload but normal serum iron and transferrin saturation has also been reported (see Differential Diagnosis section below for details). These entities can complicate the usefulness of an elevated serum ferritin in the diagnosis of hemochromatosis.

Liver biopsy:

One "gold standard" for demonstrating iron overload is that of quantitative measurement of hepatic iron content. The alternative "gold standard" is that of documenting mobilizable iron by phlebotomy until iron depletion. In addition, the histological pattern of iron deposition, particularly early in the disease process,[41, 42] may distinguish between increased iron absorption associated with HLA-linked hemochromatosis (and ineffective erythropoiesis) on the one hand and 2° iron overload associated with different liver diseases (and transfusion) on the other hand.

Until the 1980's, hepatic iron content was generally graded histologically using Perl's Prussian blue stain rather than being quantified chemically.[120] Grading systems included grade 0 = no stainable iron, grade 1 = ≤25% of hepatocytes positive, grade 2 = 25-50% hepatocytes positive, grade 3 = 50-75% positive hepatocytes and grade 4 = 75-

Figure 21: Hepatic Iron Index Distinguishes Hemochromatosis from Alcoholic Iron Overload



From: Bassett, Halliday & Powell, *Hepatology* 6:24-29, 1986

100% positive hepatocytes. Unfortunately, the mid-range grades could be observed in liver disease associated with alcohol consumption as well as with HLA-linked hemochromatosis. With the advent of standard quantitative iron measurements, distinction between alcoholic cirrhosis with increased iron stores (1,500-7,500 μg/g dry wt) and cirrhosis in HLA-linked hemochromatosis was readily apparent (>>20,000 μg/g). More sophisticated histologic grading systems have been proposed,[111] but, being inherently semi-quantitative,[121] they are unlikely to replace the "gold standard".

Hepatic iron index – In 1986, Basset *et al* introduced the concept of hepatic iron index to permit alcoholic iron excess to be distinguished from early HLA-linked hemochromatosis (<35 years) with only modestly increased iron stores.[46] They observed an increase in hepatic iron content with increasing age in HLA-linked hemochromatosis and demonstrated that calculating the hepatic iron as a function of age permitted distinction between the two diagnoses (Figure 21).[46] All HLA-linked hemochromatosis patients had an hepatic iron index (hepatic iron content in μmols/age) >2 whereas all patients with alcoholic liver disease had an index <2. Similar results were reported by other investigators utilizing the calculated index.[122] The hepatic iron index has also been used to differentiate between heterozygous and homozygous HLA-linked hemochromatosis.[50, 123] However, very early in the course of the disease, homozygotes may have hepatic iron indices <2. If other genetic or environmental factors decrease intestinal iron absorption in homozygotes (see Unusual clinical presentations section above), then they may have indices <2 even at a more advanced age. Since the abnormal value is a consequence of the genetic disease rather than a direct measurement of the gene itself, both false positive (2° iron overload) as well as false negative results may be obtained.

Genotyping:

The available evidence suggests that a point mutation, Cys282Tyr, in the *HLA-H* gene is the causative mutation in HLA-linked hemochromatosis.[4] The three independent reports confirming the initial observation[29-31] used three methodologic approaches, allele specific oligonucleotide hybridization,[29] polymerase chain reaction (PCR) amplification with restriction enzyme cleavage[30, 31] and first nucleotide change.[30] Each method permitted identification of controls, heterozygotes and homozygotes. The restriction enzyme methods each used one of two different enzymes,[30, 31] the Cys282Tyr mutation creates new *Sna*BI and *Rsa*I sites. The second mutation, His63Asp can also be detected by restriction enzyme cleavage patterns (using *Mbo*I) after PCR amplification.[30, 31] This latter methodology was also used to determine the role of HLA-linked hemochromatosis in PCT.[98]

It will now be possible to ascertain the cause of iron overload more precisely. Already, it has become apparent that there are other as yet unrecognized causes of iron overload. Thus, one patient with an hepatic iron concentration of 41,040μg/g dry wt, hepatic iron index 22.6 and cirrhosis at age 33 years has two entirely normal *HLA-H* genes (see also Hemochromatosis Gene Product section). Future studies can be specifically directed at this diagnostic dilemma.

Table VI: Diagnosis of Hepatic Iron Overload

Assay	HLA-H Hemochromatosis	Liver Disease	2° Iron Overload
Transferrin saturation	>62%	±>62%	>62%
Serum ferritin	↑↑↑	↑↑	↑↑↑
Hepatic iron	↑↑↑	↑↑	↑↑↑
Hepatic iron index	>2	<2	>2
Iron removed	>5 g	<5 g	>5 g

DIFFERENTIAL DIAGNOSIS

Although HLA-linked hemochromatosis can be diagnosed easily when the classic triad of cirrhosis, diabetes mellitus and pigmentation occurs in a familial setting with increased transferrin saturation, serum ferritin and hepatic iron index, there are other disease process that mimic some or most of these findings. The three main diagnostic categories are genetic iron overload, non-genetic iron overload and iron overload of undetermined etiology. In the future, genotyping will permit immediate identification of the HLA-linked hemochromatosis homozygote.

Genetic iron overload:

Two rare inherited diseases are accompanied by increased iron stores, hypotransferrinemia/-atransferrinemia and aceruloplasminemia.[35, 124-126] When transferrin is extremely low or absent, absorbed iron enters the portal system as non-transferrin bound iron and ≥50% is taken up by the liver.[35] When mice with this syndrome are kept viable with injections of serum containing transferrin, they eventually develop large quantities of iron in the liver, pancreas, endocrine glands and heart. In aceruloplasminemia, the iron overload is 2° to the lack of ferroxidase activity from ceruloplasmin. Ferroxidase catalyzes the conversion of Fe^{2+} to Fe^{3+}, necessary for binding to transferrin (monoferric and diferric transferrin). Loss of ferroxidase activity impairs the movement of iron from intra-cellular stores to plasma transferrin for transport. Iron accumulates in the brain as well as the liver, pancreas, heart, kidney and thyroid gland.[124]

Iron overload is also observed in 2 additional disorders with possible genetic components, African and Melanesian[108, 127] iron storage diseases. African iron overload, observed in sub-Saharan Africa, was originally considered to be environmentally based.[128, 129] Increased dietary iron derived from traditional home-brewed beer was believed to explain the disorder. More recently, a genetic effect was observed in addition to an effect of increased dietary iron.[108] The genetic effect was not HLA-linked and therefore was separate from classic hemochromatosis. A large Melanesian kindred with iron overload apparently inherited in an autosomal dominant pattern has been identified.[127] Hepatic iron contents were markedly elevated and hepatic iron indices ranged from 2.8 to 20.2, median 12.5. Once again, there was no linkage to the HLA region. The existence of these disorders suggests the possibility that similar ones may be encountered elsewhere.

Primary iron overload in African Americans[130] may be a related disorder. The finding of hepatomegaly, cirrhosis, cardiomyopathy, diabetes mellitus and/or impotence in 4 African American men was accompanied by elevated hepatic iron indices (2.3-20.2). There was no evidence for the abnormalities being 2° to ineffective erythropoiesis, blood transfusions or alcohol consumption. Further studies are needed to determine its relationship to HLA-linked hemochromatosis and the iron overload of sub-Saharan Africa.

Non-genetic iron overload:

Transfusion iron overload (Table VI) is easily separated from other causes by the history of blood transfusion. One unit of blood approximates 250mg of iron. When the number of transfusions exceeds 40 units, bone marrow reticuloendothelial stores are probably saturated and parenchymal organs/

Table VII: HLA-H Genotyping in Liver Disease Patients

Genotype	n=	Comments
Cys282Tyr Cys282Tyr	2	NASH & presumed heterozygote; hepatic iron index — 1.5, 1.3
Cys282Tyr wt	3	PCT = 1, HCV = 2; normal hepatic iron concentration
Cys282Tyr His63Asp	6	NASH = 2 & AIH, HCV, biliary cirrhosis, normal = 1 each; hepatic iron index — 0.5-1.1
His63Asp His63Asp	1	Autoimmune hepatitis (AIH)
His63Asp wt	7	HCV = 3, HBV = 1, NASH = 2, drug = 1; hepatic iron index — 0.02-0.64
wt wt	47	

From: Bacon *et al, Gastroenterology* (abstract) in press, 1997

tissues are then at risk.[131] More difficult diagnostically is the increased iron absorption in response to ineffective erythropoiesis. For example, in thalassemias and sideroblastic anemia, total body iron stores can be increased sufficiently, from enhanced absorption and a modest number of transfusions, for patients to present in a similar manner to HLA-linked hemochromatosis.[132]

Whether there is a contribution of heterozygote or homozygote HLA-linked hemochromatosis to the iron overload observed in occasional patients with anemia[133-139] cannot always be readily determined. Some of the reported patients have relatively low hepatic iron contents (<2)[133] and perhaps represent a modest increase in iron stores associated with anemia. Other patients, however, have elevated hepatic iron indices (>2).[140] It will now be possible to define the role of HLA-linked hemochromatosis precisely.

Miscellaneous iron overload:

Parenchymal liver diseases can be associated with increased iron storage (Table VI). This appears to occur more commonly in alcoholic liver disease, non-alcoholic steatohepatitis and chronic hepatitis C infection. Some of these patients may be undiagnosed homozygotes with both HLA-linked hemochromatosis and another etiology for liver disease. Bacon and co-workers have genotyped 66 patients with chronic liver disease (Table VII).[141] Two patients were homozygous for the Cys282Tyr mutation. They had previously been considered heterozygotes (increased hepatic iron but index <2). In 17 others, either Cys282Tyr or His63Asp was present on one or both chromosomes. Except for one patient with PCT, there was no obvious consequence of these mutations.

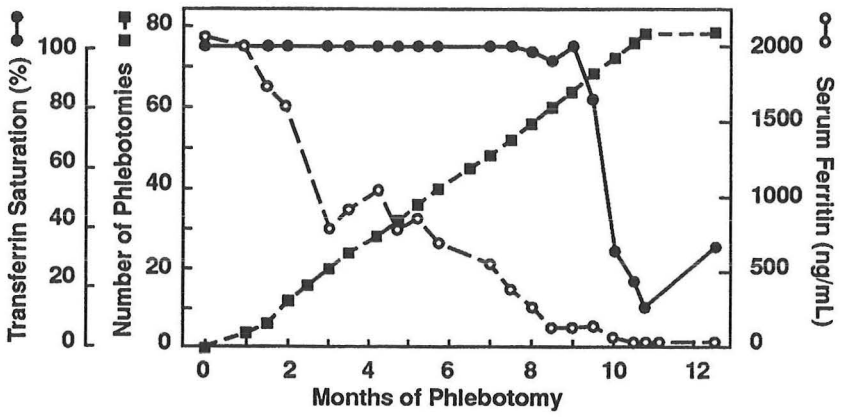
A syndrome of hyperferritinemia and increased hepatic iron content with normal transferrin saturation has recently been described.[142] Hepatic iron indices were >1.9 in 22 of 65 patients. Almost all the patients (95%) had one or more metabolic disorders (obesity, hyperlipidemia, abnormal glucose metabolism and hypertension). The iron overload was mild to moderate in severity (mobilizable iron <10g and no reported hepatic fibrosis/cirrhosis). Additional studies are needed to determine the frequency and importance of this entity.

Neonatal hemochromatosis is a descriptive diagnosis of the prenatal onset of iron overload involving the liver, heart, endocrine glands and kidney with sparing of the reticuloendothelial cells.[143-145] Hepatic and multi-organ failure with rapid demise generally follow. Similar changes have been observed in infants with a primary genetic defect in bile acid synthesis.[146] Currently, it is not certain whether this represents one condition or, more likely, a heterogenous group of familial and environmental disorders, each characterized by a disturbance in fetal/neonatal iron metabolism. None of 4 cases had mutations in *HLA-H*.[29]

Treatment and Prevention

HLA-linked hemochromatosis is a disorder of increased total body iron stores, resulting from failure to regulate the absorption of iron. There is no physiologic pathway for iron removal except for losses in desquamated cells of the skin and gastrointestinal tract and the normal losses of menstruation and pregnancy. Treatment consists of removal of excess iron. The simplest method

Figure 22: Response to Phlebotomy Therapy for Hemochromatosis



From: Edwards *et al*, *Ann Int Med* 93:519-525, 1980

is by blood-letting (phlebotomy). Alternatively, iron can be bound to iron chelating compounds and excreted in the urine.

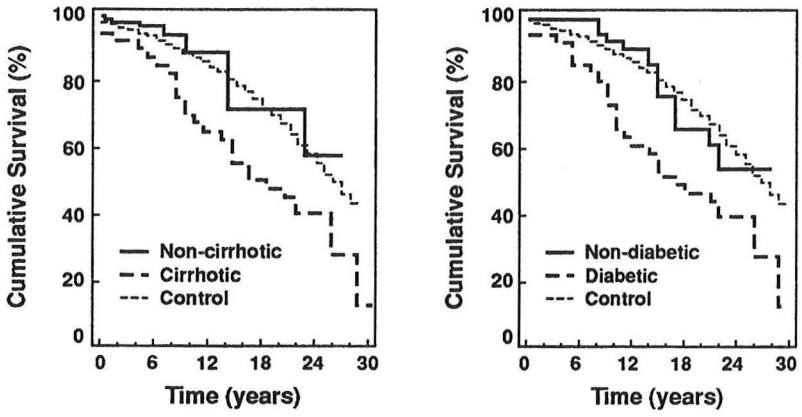
Phlebotomy:

Each unit of blood contains ~250mg of iron in hemoglobin. Following phlebotomy, the transient fall in hemoglobin/hematocrit is followed by an increase in bone marrow erythropoiesis with resultant return to normal. The process of red cell formation uses available iron from marrow stores. Body iron stores equilibrate to maintain marrow iron availability, releasing iron from cellular ferritin, with resultant decreases in tissue iron content. Phlebotomy can be continued on a weekly basis without developing anemia before iron depletion in most patients. The initial measurable response is a decrease in serum ferritin (Figure 22). Not until the ferritin level has fallen below normal does the serum iron decrease and transferrin saturation diminish. Finally, with removal of all mobilizable iron (can exceed 40g = 160 units of blood), an iron deficiency state can ensue. With cessation of phlebotomy, the serum iron and transferrin saturation start to rise again. Regular interval phlebotomy (every 2-4 months) may then be needed to maintain iron balance. Alternatively, patients can be monitored and phlebotomy re-introduced when the serum ferritin exceeds the normal range (350ng/ml in men and 200ng/ml in women).[147] Administration of erythropoietin with phlebotomy may increase the efficiency of iron removal in patients with co-existent anemia unrelated to iron.[148]

The history of phlebotomy, detailed by Crosby,[149] includes the proposal by a number of investigators that iron removal by blood-letting may be beneficial. He credits Davis and Arrowsmith with the introduction of phlebotomy in 1947 and providing the formal proof of principle in publications in 1950-1952.[150,151] Since then, this approach has been the mainstay of therapy once hemochromatosis is diagnosed. When successfully applied to non-cirrhotic individuals, normal survival ensues (Figure 23).[51] Removal of iron can be accompanied by a decrease in established hepatic fibrosis (observed in 42 of 185 patients) but more often merely prevents progression (141/185).[51] The deterioration in the extent of fibrosis in two of the 185 patients most likely represents sampling error or the existence of another source of hepatic injury.

Treatment of cirrhotics – Phlebotomy therapy is also useful in cirrhotic liver disease although a normal life-span may not be attained. Markers of portal hypertension can diminish, for example esophageal varices decreased or disappeared over a 2 to 10 year period in 7 of 13 patients with HLA-linked hemochromatosis and no other cause of liver disease.[152] In contrast, varices remained unchanged in 15 patients and worsened in one of a total of 17 patients with concomitant viral liver disease. There is the potential for improvement in cardiac and endocrine manifestations with phlebotomy[79] but in reality this is uncommon.[111,153] The arthropathy is unchanged.[93,154] In the United States, blood removed at prescribed phlebotomy is generally discarded. In the United Kingdom, these units may be used for External Quality Assessment Schemes that require large volumes of human serum.[155] In Canada, the Canadian Red Cross permits otherwise healthy subjects with HLA-linked hemochromatosis to become regular volunteer blood donors, a

Figure 23: Normal Survival in Non-cirrhotic, Non-diabetic Hemochromatotic Patients with Treatment



From: Niederau *et al*, *Gastroenterology* 110:1107-1119, 1996

particularly enlightened policy.

Chelation therapy:

In patients with iron overload 2° to transfusion therapy, phlebotomy is not an option. In addition, patients with HLA-linked hemochromatosis and an unrelated anemia or critical cardiac function may not tolerate phlebotomy. Chelation therapy is the only alternative treatment at this time. Iron bound to deferoxamine (desferrioxamine) is excreted in the urine. Deferoxamine is poorly absorbed after oral administration and must be given parenterally for effectiveness. Administration of a single dose of deferoxamine (750mg) intravenously or subcutaneously results in comparable urinary iron excretion over the ensuing 24 hour period.[156] Continuous subcutaneous infusion of 750-2,250mg of deferoxamine is 80-90% as effective as intravenous administration.[156] Iron excretion rates of 500-600mg/month (mean) can be achieved with chronic subcutaneous therapy. Local toxicity (pruritus, erythema, swelling) is encountered but the main problem is that of compliance with the continuous subcutaneous regimen. Intra-peritoneal deferoxamine is also efficacious.[157]

In the management of patients with thalassemia major, introduction of regular blood transfusions permitted the prevention of anemia and bone marrow expansion, with normal growth and development. However, the price was that of iron overload with a propensity for lethal cardiac disease. With the development of safe and effective chelation therapy, survival without the complications of 2° iron overload is now possible.[158] Orally effective chelation therapy is the next awaited development.

Outcome:

In cirrhotic patients, survival is significantly decreased compared to the normal population even if treated.[51] Long-term survivors have lower levels of mobilizable iron on average than those who succumb despite complete removal of iron (19.4 ± 1.7g compared with 29.1 ± 2.6g).[51] In their large series of prospectively followed patients, Niederau *et al* observed that causes of death before iron depletion included complications of cirrhosis (9/34), liver cancer (either hepatocellular carcinoma or cholangiocarcinoma, 4/34), heart failure and diabetes.[51] Of those dying after complete removal of iron, 15 of 35 had hepatocellular carcinoma or cholangiocarcinoma. In all, there were 16 patients who died with hepatocellular carcinoma and 3 with cholangiocarcinoma, a rate of primary liver cancer 119-fold higher than in controls. Cardiomyopathy (5 patients) and diabetes (4 patients) were 14 times more frequent causes of death and cirrhosis (14 patients) was 10 times more frequent.[51] Deaths from neoplasms other than primary liver cancer (8 patients) were not increased in frequency nor were deaths from myocardial infarction or other circulatory system diseases.

Transplantation:

The alternative therapy for patients at risk of dying from complications of cirrhosis is that of transplantation. If transplantation is carried out before the development of hepatic malignancy, it could also serve as an effective preventive therapy for this major complication. Thus, orthotopic liver transplantation, if successful, could potentially have

precluded the deaths of 33 of the 69 patients in the series of Niederau *et al.*

Six patients with iron overload and findings consistent with HLA-linked hemochromatosis underwent orthotopic liver transplantation between 1982 and 1988.[159] Each patient had undergone long-term phlebotomy and/or chelation therapy (mean 7 years, range 3 to 12 years). One died soon after from hepatic artery thrombosis, the other 5 were well with follow-up from 6 months to 5½ years. These favorable results were not invariably obtained. Of 9 patients transplanted at one center between 1988 and 1992, there were 5 survivors (3 to 25 months post-transplant).[66] The major difference between the two groups was that of prior recognition of the disease and institution of appropriate therapy before the need for transplantation arose. Only 2 of the latter 9 patients were diagnosed pre-transplant,[66] compared with 6 of 6.[159]

Orthotopic liver transplantation has also provided invaluable proof of the role of the liver (innocent bystander) in the pathogenesis of this disease. Thus, inadvertent transplantation of livers from donors with unrecognized iron overload is followed by gradual depletion of iron from the donor liver.[160, 161] In the one exception to this observation, the recipient demonstrated increased iron absorption and was probably an unrecognized heterozygote or even homozygote.[162] In addition, Farrell *et al* observed hepatic iron accumulation in the donor liver following transplantation, further evidence that the liver is not the primary organ determining the disease expression.[66]

Post-transplant survival of hemochromatosis patients in the United States is decreased compared with that of other diseases.[163] Survival at 1 year and 5 years in Medicare patients was 79% and 59% respectively overall whereas in patients with iron overload it was decreased to 54% and 43% respectively. Similar findings have been reported in a recent review.[164] The available evidence indicates that this poor outcome is related to failure of adequate pre-transplant diagnosis and treatment. Based on the patients followed by Niederau *et al*, even cirrhotic patients would be expected to have survival rates comparable to those of patients with other causes of liver disease. When hemochromatosis is diagnosed and treated before the need for transplantation arises, such as in the initial group reported from the University of Pittsburgh and in patients transplanted in Australia (cited in reference 165), outcomes after transplantation are comparable to those of other diseases.

Prevention:

The goal for all physicians is the prevention of morbidity and mortality related to disease. This may be best achieved for HLA-linked hemochromatosis by early diagnosis and complete avoidance of any tissue/organ damage from iron overload. To this end, a number of investigators have used model systems to examine the costs, effects and benefits of screening for this disorder. The rationale is straight forward: 1) HLA-linked hemochromatosis is the commonest inherited disease in Caucasian populations; 2) survival is normal if diagnosed and treated before the advent of hepatic disease; 3) treatment for asymptomatic subjects is inexpensive, effective and readily available. If the screening identifies patients with 2° iron overload, they may also benefit from recognition of the disorder and institution of appropriate therapy to remove increased iron stores.[131, 158]

The general approach to screening is that of using a simple, reliable and cost-effective test to identify those with increased likelihood of the disease. Positive results are then followed up by confirmatory tests and initiation of therapy. Transferrin saturation and the measurement of unsaturated iron binding capacity have been used in model systems.[166-168] In 3 separate studies using somewhat different model systems, the authors found cost-effectiveness ratios in favor of screening.[166-168] Variables with impacts on the models were: 1) prevalence of the disease, probability of developing disease manifestations and the discount rate;[166] 2) estimated cost of disease treatment and screening test specificity;[167] and 3) prevalence of the disease and cost of the screening test.[168]

This theoretical approach has been tested in practice.[169] Transferrin saturation (fasting) was used to screen 3,977 consecutive men ≥30 years presenting for routine health maintenance (1,974 Caucasian, 1,148 African-American, 251 Asian and 474 other ethnic background). Subjects with a transferrin saturation ≥62% were followed with additional iron chemistry evaluations (repeat serum iron and total iron binding capacity, serum ferritin), estimations of hepatic and endocrine function/integrity (ALT, AST, alkaline phosphatase, bilirubin, albumin, prothrombin time, fasting glucose, serum

testosterone) and hematologic parameters (complete blood count). In 40 subjects, the initial screening test was positive. All 36 available for follow-up were asymptomatic with normal physical examination and blood chemistry values. Only 14 subjects had persistently elevated transferrin saturation, 12 were biopsied. Hemochromatosis was confirmed in 8 patients (hepatic iron index 1.9-17.1), while 4 had minimal iron loading (hepatic iron index 0.7-1.2).[169] One patient was Asian (HLA-type unknown) and the remaining 7 were Caucasian. Three additional asymptomatic relatives were identified.

Screening charges, calculated from a representative fee schedule were $49.02 per patient. The major cost was that of the initial screening ($40.88, 83%), the remainder being generated from confirmatory tests including liver biopsy ($2.65 per patient screened) and evaluation of low iron saturation, encountered in 172 patients ($5.49 per patient screened). As carried out, the cost was ~$17,000 per case identified. If screening had been confined to the Caucasian population, this would have been halved and only 1 Asian patient with iron overload missed. The identification of 7 presumed homozygotes for HLA-linked hemochromatosis in 1,974 Caucasian males (1:282) is similar to predictions. A second study identified far fewer patients (4 in 12,258 patients)[170] perhaps because the initial screening test, serum iron is less sensitive as well as being less specific.[114]

Conclusions

HLA-linked hemochromatosis is the commonest inherited disease in Caucasians. There is unregulated transfer of iron from the intestinal epithelial cells to the body and iron overload eventuates. Iron transport proteins become saturated and iron stores increase, reflected by an increase in serum ferritin. All the proteins involved in inter-cellular iron transport (transferrin/transferrin receptor), iron storage (ferritin) and regulation of intra-cellular iron content (iron regulatory proteins) are normal and normally regulated. There is a point mutation in an HLA class I-like molecule, termed HLA-H, that confers the phenotype of inappropriate iron absorption. The precise mechanism whereby mucosal iron transfer is increased is unknown. The retained iron probably damages tissues/organs by the generation of reactive oxygen species and subsequent lipid peroxidation. Progressive iron deposition results in hepatic fibrosis and cirrhosis, dilated cardiomyopathy, diabetes mellitus, hypogonadotrophic hypogonadism, arthropathy and skin pigmentation. Survival is diminished by deaths from complications of iron-mediated liver, cardiac and pancreatic damage. Treatment consists of removal of excess iron by regular phlebotomy or chelation therapy and is effective at improving outcome. Orthotopic liver transplantation can improve survival in those with major complications from hepatic cirrhosis that do not respond to phlebotomy. Early diagnosis, achieved by screening persons at risk for the disease, is predicted to prevent morbidity and mortality associated with this condition.

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