

Hemochromatosis and Iron Needs

[Seventeenth Marabou Symposium: Diet And Genetic Interactions]

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Abstract

Although iron is an essential dietary requirement, the amount absorbed by the body is well regulated and depends on body iron stores and on dietary iron availability. There is very little iron excreted under normal conditions. Iron deficiency is a worldwide problem but iron overload, as seen in the inherited disease, hemochromatosis, is a major cause of morbidity in some Caucasian populations. This is a problem particularly where there is an adequate dietary iron intake and especially in males. A mutation has recently been described in an MHC Class I-like gene (HFE) that encodes for a protein (HFE) of 343 amino acids. The molecule contains a signal sequence peptide-binding region,  $\alpha$ 1 and  $\alpha$ 2 domains, and an immunoglobulin-like  $\alpha$ 3 domain, in addition to a transmembrane region and a small cytoplasmic tail. It is a candidate gene for

hemochromatosis. Several possibilities as to the function of this gene and the corresponding protein have been suggested but none has yet been confirmed. The mutation has been detected by several different groups in 80%-100% of subjects with the disease. However, in one study, 18%-20% of patients with the mutation did not exhibit significant iron overload. The discovery of this gene has important implications for both clinical studies and the elucidation of the pathways of iron metabolism.

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## Introduction

Iron is an essential requirement of virtually all living organisms. Indeed, if iron had not existed on the earth, it is unlikely that life as we know it would have evolved. It is also true that an appropriate iron balance must be maintained for survival. Iron deficiency is one of the major causes of morbidity and mortality throughout the world, especially in less developed areas.<sup>1,2</sup> It is likely that any major inborn error resulting in severe iron deficiency in utero is incompatible with life. Hence, little is known of genetic disorders relating directly to physiologic iron metabolism.

Some refractory anemias result from genetic abnormalities not directly related to iron metabolism but are accompanied by secondary problems, such as iron-overload.

Excess iron is damaging, as demonstrated by the disease hemochromatosis. This is one of the most common inherited disorders seen in Caucasian populations in Australia, Europe, and North America, with an estimated disease frequency of 1:300 to 1:400. The inappropriate increase in intestinal iron absorption that is characteristic of hemochromatosis results in the deposition of iron in parenchymal cells, leading eventually to tissue damage and functional impairment of the organs in which the excess iron occurs, e.g. the liver, pancreas, heart and the pituitary gland. This excessive iron deposition may also occur as a consequence of increased iron absorption resulting from ineffective erythropoiesis

such as that seen in thalassemia, also an inherited disease. The primary defect in thalassemia, however, is not in the iron cycle but rather in the hemoglobin molecule.

Although the association between cirrhosis, deposition of pigment in the liver, and diabetes mellitus was first recognized by Troisier in 1871,<sup>3</sup> it was not until 1935 that the English physician Sheldon first emphasized that hemochromatosis

probably resulted from an inborn error of iron metabolism.<sup>4</sup> Until recent times, a diagnosis of hemochromatosis was made only in patients who presented with clinical signs and symptoms of disease. These included skin pigmentation, hepatomegaly, and diabetes. Loss of libido and testicular atrophy were common and cardiac manifestations occurred in 5% to 15% of symptomatic patients.<sup>5</sup> The presence of such tissue injury is no longer essential for the diagnosis to be made.

In 1975, Marcel Simon first demonstrated a significant association between the HLA-A3 locus and hemochromatosis.<sup>6</sup> This was confirmed in succeeding years<sup>7-9</sup> and it is now known that the disease is inherited as an autosomal recessive trait with the susceptibility locus tightly linked to the HLA locus on chromosome 6. The gene frequency has been estimated to be approximately 1 in 10 with a homozygote frequency in Caucasian populations of approximately 1 in 300.<sup>10</sup> It took more than 20 years from Simon's landmark discovery until a

putative gene responsible for the development of this iron overload disorder was finally identified in a paper by Feder et al in August 1996.<sup>11</sup>

An additional advantage of the Simon observation was that it enabled the use of HLA typing to assist in diagnosis of the disease within the family of an affected subject. Diagnosis of hemochromatosis is now frequently made in subjects who remain asymptomatic. In such subjects, elevated serum iron, serum transferrin saturation, and serum ferritin concentration may be detected by serological tests. A liver biopsy followed by chemical determination of the hepatic iron concentration then permits the calculation of an hepatic iron index (hepatic iron concentration in  $\mu\text{mol/g}$  dry weight  $\times$  age in years).<sup>12,13</sup> An index greater than 1.9 is considered abnormal and in the absence of secondary causes of iron overload such as iron-loading anemia, usually represents homozygous subjects. Heterozygotes, as defined by HLA typing within families, do not accumulate iron to the same extent as homozygotes but approximately 25% of such heterozygotes may show some biochemical abnormality such as an increase in transferrin saturation.<sup>10,14,15</sup> HLA typing has been used to predict the likelihood of a sibling developing the disease in the future and early diagnosis has permitted intervention by phlebotomy to remove excess iron stores, thus preventing tissue damage.<sup>16</sup> For example, the relative risk of development of hepatocellular cancer in patients with iron overload who present with cirrhosis is 200.<sup>17</sup> If phlebotomy therapy is instituted in patients who are precirrhotic, the removal of excess iron appears to prevent the development of cirrhosis and of subsequent hepatocellular carcinoma.<sup>16</sup> In the absence of a known gene for the disease, the use of HLA typing has allowed all first degree relatives to be offered screening for phenotypic expression of the disease, and the hepatic iron index has provided a useful indicator of such expression.<sup>18</sup> As a result, the prognosis for survival has improved so that the survival curve, when the disease is detected before cirrhosis has developed, now approaches normal.<sup>19,20</sup>

#### Normal Iron Metabolism

It is beyond the scope of the present paper to discuss human iron metabolism in depth, which has recently been reviewed.<sup>21</sup> The discovery in the 1980s of iron-regulatory elements in the mRNA for a number of proteins, notably ferritin and the transferrin receptor, revealed much concerning the control of the level of expression of those proteins. Nevertheless, despite the enormous amount of work devoted to the study of iron metabolism in both human and animals, the major pathways of iron metabolism and especially of iron uptake into the body, are still poorly understood. We do know that the amount of iron within the human body is controlled largely at the point of entry—the intestinal mucosa—and that there is no major excretory pathway for iron in man. Hence, any increase in iron intake by the body, either by a prolonged increase in iron absorption or by the administration of parenteral iron, in the form of transfusions for example, produces an increase in body iron stores unless the iron is removed by pathologic means, such as blood loss. The biochemical mechanism by which the body controls the entry of iron to maintain adequate but not excessive iron stores has remained an enigma.

It is still unclear whether the mechanism for control of iron uptake resides in the intestinal cell itself or in some internal messenger that relays information to the gut. Such a messenger must respond to body iron stores and to erythropoietic activity, both of which are known to affect iron absorption. Iron losses from the skin, gastrointestinal tract, and genitourinary tract amount to approximately 1 mg/day in adult males and 2 mg/day in premenopausal females. In a state of

iron balance, a similar amount of iron is absorbed daily. However, both environmental and genetic factors are known to influence the amount of iron absorbed.<sup>1</sup>

## Factors Influencing Iron Stores

### Environmental factors

Age and gender. Iron stores vary according to age and gender.<sup>22</sup> Serum ferritin concentration is an indicator of body iron stores in the absence of inflammation, liver disease, or some malignancies. It rises in all subjects with age, albeit more slowly in women than in men. In women, serum ferritin levels usually remain low until after the menopause and thus, heavy iron overload due to hemochromatosis usually occurs earlier in men than in women.

Blood donation. Any loss of blood depletes iron stores. Frequent blood donations cause a marked decrease in iron stores in both men and women, but more readily result in iron deficiency in women, presumably because they have lower initial iron stores that are more easily depleted.<sup>22-24</sup> Frequent blood donation may delay or even prevent the accumulation of excessive iron in individuals with hemochromatosis.

Dietary iron intake. It is very difficult to ascertain to what degree dietary iron content influences iron stores. Most studies have examined single populations in whom dietary iron content is relatively uniform. Iron is absorbed as heme (found in meat) and nonheme iron. Heme iron is better absorbed than nonheme iron and also improves the absorption of nonheme iron in the diet. The mechanisms of these interactions are not well understood. Nonheme iron accounts for more than 90% of total iron in the average Western diet, which contains approximately 90 mmol of iron (5 mg) per 1000 kcalories. Normal males consuming 3000 kcalories per day obtain approximately 270 mmol (15 mg) of iron per day, of which 9-27 mmol (0.5-1.5mg) is absorbed. The possible range of iron absorption from the normal diet is limited (less than 9 mmol  $\pm$  0.5 mg<sup>93</sup>; daily in iron-replete subjects to approximately 72 mmol  $\pm$  4 mg<sup>93</sup>; in iron-deficient subjects). The availability of dietary iron is modified by other factors, including dietary fiber, tannins, phytates, and drugs such as cholestyramine and tetracyclines, which result in decreased iron absorption and by vitamin C and heme iron, which increase iron absorption. The bioavailability of iron in various diets has been extensively studied.<sup>2</sup>

Most bioavailable iron is present in meat; the variation in meat consumption between different populations certainly contributes to varying iron stores. In Sweden, meat consumption is reported to be only 54% of that of Australia and in the UK only 68%.<sup>25</sup> The serum ferritin concentration reflects body iron stores in the absence of inflammation. In the Australian population, diet has been shown to have a significant effect on the serum ferritin concentration of women.<sup>21-24</sup> Variations in the diet are also likely to influence the rate of iron accumulation in hemochromatosis, but a simple reduction in dietary intake is unlikely to totally mask the expression of the disease. The identification of the gene will allow further investigation of the degree of phenotypic expression in those homozygous for the gene.

Supplemental oral iron intake. Few data have been published on the effect of continued iron supplementation once iron deficiency has been corrected. The

question as to whether or not the ingestion of pharmaceutical doses of iron over many years can overcome the normal barriers to excessive iron absorption and lead to inappropriate iron accumulation has not really been resolved, although this scenario seems likely. The discovery of the hemochromatosis gene will also assist in answering the question as to whether one or two such genes are required or whether excessive iron can accumulate in the absence of this gene under conditions of prolonged high intake.

Pathologic blood loss and malabsorption. Despite the resultant compensatory increase in iron absorption, regular blood loss of more than 10 mL of blood daily is likely to lead to iron deficiency, especially in women. People with hemochromatosis may have an increased iron absorption of more than 4 mg per day, but even they may become iron deficient if losses are prolonged and severe. Diseases such as inflammatory bowel disease and celiac disease, in which iron deficiency is common, may mask the expression of hemochromatosis in subjects who are homozygous for the disease. The intake of some drugs, such as cholestyramine, and some antibiotics such as tetracyclines when taken for a prolonged period may also reduce iron absorption and hence, deplete iron stores.<sup>2</sup>

#### Genetic Factors

In many inherited conditions there is a close relationship between genotype and phenotype. In cystic fibrosis, for example, different mutations may account in part for the variable phenotypic expression of the disease.<sup>26</sup> Concordance of disease expression between affected siblings provides evidence that genetic factors are significant determinants of disease expression. In a study of disease expression in siblings with hemochromatosis,<sup>24</sup> a wide range of hepatic iron concentration (32 to 833 mmol/g dry wt) was found and the hepatic iron index ranged from 1.65 to 14.4. There was no evidence that these differences were due to differing exposure to those environmental factors known to influence iron stores. However, a highly significant correlation for both hepatic iron concentration and hepatic iron index was found between siblings of the same sex. Of the 22 same-sex sibling pairs studied, the hepatic iron concentration of one sibling was less than 50% of the other in only three pairs. In each of these three pairs the discordance could be explained by either multiple previous blood donations or by HLA non-identity, thus supporting the hypothesis that the extent of hepatic iron loading is principally determined by genetic factors. Further recent studies by the same group<sup>27</sup> recently showed that Australian patients who were homozygous for an ancestral haplotype for hemochromatosis accumulated more liver iron, as measured by the hepatic iron index, than did affected patients who did not carry two copies of this haplotype.

Similar findings have also been reported in several other studies, indicating that genotype appears to be an important determinant of phenotypic expression in hemochromatosis. It was interesting to note that in the Australian study, the frequency of iron deficiency was much less in female heterozygotes than in the general population. Such investigations suggest a selective advantage conferred by the presence of one copy of the hemochromatosis gene in protecting women against iron deficiency. Female homozygotes could also benefit during their reproductive years. All female groups therefore would have a survival and reproductive advantage. The majority of males carrying either one or two genes for the disease survive to reproductive age so that the selective advantage conferred by protection from iron deficiency could be an important factor determining the prevalence of the disease in the community.

#### The Putative Biochemical Defect

The major unresolved issues regarding hemochromatosis still relate to the site and nature of the primary biochemical defect. The most likely candidates for the site of the defect are the intestinal cells, the liver, and the cells of the reticuloendothelial system. However, it is tempting to speculate that the hemochromatosis gene involves a generalized iron transport abnormality or perhaps a regulatory defect in a transporter. The arguments for and against such defects prior to the report of a putative gene for the disease have been examined in an earlier publication.<sup>21</sup> The implications of the recent paper by Feder et al.<sup>11</sup> are discussed below.

#### The Genetics of Hemochromatosis

Despite the fact that the gene responsible for hemochromatosis was not known, its location on the short arm of chromosome 6 (6p) in close proximity to HLA-A has allowed the successful tracking of the gene in affected pedigrees. The pattern of inheritance within a particular family can be traced by HLA typing of first degree relatives of the proband.<sup>21</sup> Affected siblings of the proband usually have two HLA haplotypes identical to those of the proband (homozygous), whereas unaffected siblings have one or neither haplotype identical to the proband.<sup>7,9,15,18</sup> In siblings resulting from a homozygous/heterozygous mating, affected individuals share the HLA haplotype from the unaffected (heterozygous) parent and may inherit either haplotype from the affected (homozygous) parent.<sup>8,28</sup>

In some previous studies it appeared that in certain populations, the majority of relatives who were designated homozygous in this manner would eventually exhibit full clinical and biochemical expression of the disease. However, as already mentioned, expression depends on other factors. In a recent Australian study,<sup>8</sup> 50 homozygous relatives were defined by HLA studies. Of these, 47 relatives expressed the disease during a follow-up period of some 8 years. In contrast, those designated as heterozygotes did not demonstrate a progressive increase in iron stores of the order seen in homozygotes, although some did present with some minor biochemical abnormalities. Where putative heterozygotes appeared to develop progressive iron overload, the results were accounted for on the basis of either chromosomal recombination or homozygous/heterozygous matings resulting in the misclassification of homozygotes as heterozygotes.<sup>6,7,9,15,18</sup>

Recent advances in the techniques of molecular genetics have allowed much more precise study of hemochromatosis patients and of the location of the gene. The primary technique used is that of positional cloning-cloning on the basis of chromosomal location. The first stage in positional cloning is to define more closely the region of the chromosome that contains the gene. For this, linkage analysis is used, which predicts the statistical likelihood that marker loci are inherited together within pedigrees. The recent identification of numerous short tandem repeat sequences (microsatellites) in DNA has provided a large number of highly polymorphic markers that have greatly facilitated such studies.

A recent Australian study<sup>29</sup> indicated a clear association between hemochromatosis and specific alleles at HLA-A and D6S105, namely HLA-A3 and D6S105 allele 8. Eighty-two unrelated hemochromatosis patients and 82 unrelated healthy controls were studied; D6S105 allele 8 was present in 93% of the patients and 21% of controls. These results indicated that D6S105 was the closest marker to the hemochromatosis gene reported up to that time. Close association between D6S105 and the hemochromatosis gene was subsequently confirmed in patients from Italy, France, and the United Kingdom. These studies also indicated that the gene

appeared to be telomeric to HLA-A on the chromosome and perhaps not as close as had previously been considered.

More recently a haplotype analysis of chromosomes from 26 hemochromatosis pedigrees containing multiply affected subjects was also carried out in Australia.<sup>30</sup> Several polymorphic markers were examined: HLA-A (serological) and microsatellites D6S248, D6S265, HLA-F, and D6S105. All of these markers showed a highly significant allelic association with hemochromatosis without evidence of recombinations between the disease locus and the marker. A predominant ancestral haplotype allele 5-1-3-2-8 (marker order D6S248, D6S265, HLA-A, HLA-F, and D6S105) was exclusively associated with hemochromatosis with a relative risk of 903. This haplotype was present in 33% of the 64 affected chromosomes, providing strong evidence for a common mutation associated with hemochromatosis in Australian patients and the probable introduction of hemochromatosis into the population on an ancestral haplotype. As discussed above, recent evidence also suggested that hemochromatosis patients with two copies of the ancestral haplotype showed significantly more severe expression of the disorder.<sup>27</sup> Genetic mapping suggested a distance of 1 to 3 centimorgans telomeric to HLA-A with an estimated physical distance of 3 to 4 megabases.<sup>30,31</sup> A highly significant association of the disease with the satellite marker D6S1200 was then published.<sup>32</sup>

That microsatellite marker lies 700 kilobases telomeric to D6S105; thus evidence was accumulating that in order to find the gene one should be looking telomeric to all of the previous markers.

The task of gene identification has been made easier by the recent development of yeast artificial chromosomes (YACs), which are capable of carrying segments of human DNA up to one or two megabases in length. The final stage of positional cloning involves screening the cloned region for coding sequences and determining

which, if any of them, represents the hemochromatosis gene itself, by studying gene expression and by the detection of mutations.

#### The Putative Gene

In August 1996, a paper was published by Feder et al.,<sup>11</sup> describing a novel major histocompatibility complex (MHC) Class I-like gene that was mutated in patients with hereditary hemochromatosis. They used linkage disequilibrium and haplotype analysis to identify a 250 kilobase region more than 3 megabases telomeric to the MHC region that was identical in 85% of patient chromosomes. Within this region, using cDNA selection and genomic sequencing, they identified a gene related to the MHC Class I family. They termed it HLA-H but as this term was already in use, it was renamed the HFE gene.

Analysis of sequences identified three novel genes and 12 histone genes within a 250kb region. All 15 genes identified between D6S2238 and D6S2241 were analyzed for sequence variation by comparing two patients who were homozygous for the ancestral haplotype to two controls. Two of the 15 genes contained base differences predicted to result in amino acid alterations. The only nucleotide change consistent with the ancestral mutation occurred in an MHC Class I-like gene and was a G-A transition at nucleotide 845 of the open reading frame that results in a cysteine to tyrosine substitution at amino acid 282. This Cys282Tyr (C282Y) missense mutation occurred at a highly conserved residue involved in intramolecular disulphide bridging in MHC Class I proteins and could, therefore, disrupt the structure and function of such proteins. This mutation was detected in 85% of all hemochromatosis chromosomes and in only 3.2% of the control chromosomes, giving a carrier frequency of 6.4%. Most of the patients studied

(148 of 178) were homozygous, 9 were heterozygous, and 21 carried only the normal allele. A second mutation within this gene was a C-G change in exon 2 that resulted in the substitution of histidine to aspartic acid at position 63. However, this was also present in control chromosomes. The cDNA clone of interest was 2.7 kilobases with an open reading frame of 1029 bases encoding a predicted protein of 343 amino acids. The protein appeared to be most similar to MHC Class I gene products, including HLA-A2 and a nonclassical Class I-like molecule such as HLA-G. There was also some similarity with the human FC receptor. The molecule contains a signal sequence peptide binding region, [alpha]1 and [alpha]2 domains and an immunoglobulin-like [alpha]3 domain. It also contains a transmembrane region and a small cytoplasmic tail (Figure 1).

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Figure 1. Hypothetical model of the HFE protein. The approximate locations of the Cys282Tyr and His63Asp mutations are indicated. (After Feder et al.,<sup>11</sup> with permission.)  
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#### Possible Functions of the HFE Protein

One of the most important conserved structural features of MHC Class I molecules, which is also conserved in the HFE protein, consists of the four cysteine residues that form disulphide bridges in the [alpha]2 and [alpha]3 domains. The correct conformation of the [alpha]3 domain is needed for noncovalent interaction with [beta]2 microglobulin and the correct cell surface presentation. One of these conserved cysteines is altered in the C282Y mutation. If the cysteine is mutated at the corresponding position in the disulphide bond of the equivalent protein in the H-2L mouse, it eliminates intracellular transport of the protein from the endoplasmic reticulum to the plasma membrane. By analogy, Feder et al.<sup>11</sup> suggested that the observed mutation may have similar consequences. Support for the existence of a defective MHC Class I-like gene causing hemochromatosis has also been presented in studies of [beta]2 microglobulin

"knock-out" mice. Such mice display a progressive hepatic iron overload.<sup>33</sup> It is possible that the relevant protein may internalize and/or recycle ligand via receptor-mediated pathways. If the ligand was an iron-binding protein, the protein might, in normal circumstances, act in a negative fashion to limit the iron-uptake process. If the mutation then altered the gene product in such a way that the control of ligand uptake, and consequently of iron uptake, was removed, one could account for increases in iron stores.

A second possibility canvassed by Feder et al.<sup>11</sup> is a role in signal transduction

whereby the HFE protein senses plasma iron levels and regulates appropriate genes or products that control the rate of iron transfer. The protein could also act more indirectly through association with components of the immune system. Such interactions remain hypothetical and functional studies are eagerly awaited.

In a recent paper Parkkila and co-workers<sup>34</sup> studied the expression of the HFE protein in various tissues, using an antibody generated to the C-terminal peptide. They found that the protein was highly expressed in crypt cells of the gut in an intracellular and perinuclear distribution, but not in villus tip cells. It was expressed in the canalicular membranes in liver but was not expressed in brain. Such expression would fit very well with a protein having a role in iron absorption or iron transport. However, other studies have indicated a more general distribution of the protein. Further studies using other

antibodies are therefore needed.

#### Expression of the Putative Gene in Patient Groups

Several investigators 35,36,37 have now analyzed patient material for the C282Y mutation. Beutler et al 35 reported 82% of 147 patients as homozygous, 7% as heterozygous, and 11% who did not carry the mutation. They did not examine pedigrees. An Australian series of 181 individuals from 26 well-characterized pedigrees revealed the mutant gene on every affected chromosome and the nonmutant form on all unaffected chromosomes.<sup>36</sup> A further study (Crawford et al, personal communication) of hemochromatosis patients revealed that when 300 subjects from 101 pedigrees were tested for the C282Y mutation in the HFE gene and grouped according to whether they were homozygous or heterozygous for the mutation or homozygous normal, all adults previously diagnosed as homozygous or heterozygous carried at least one C282Y mutation. However, of the 125 subjects who were homozygous, only 82% met the clinical criteria for the diagnosis of iron overload. In addition, in 11 of 173 subjects who were heterozygous for the mutation, iron indices were within the range previously regarded as indicative of homozygosity. All showed a modest increase in iron stores and no evidence of organ dysfunction. Thus, in Australia approximately 18% of subjects (17 females and 5 males) who were homozygous for the C282Y mutation did not express iron overload sufficient to meet the current diagnostic criteria and 7% of subjects heterozygous for the mutation presented with modest iron overload in the range previously diagnosed as homozygous. Of the 17 females, 12 were premenopausal and 2 postmenopausal women had reasons for not accumulating iron, including inflammatory bowel disease and multiple pregnancies. Nevertheless, the predominance of women who did not express the disease may imply not only that iron loss may provide an explanation but also the possibility of hormonal regulation of some aspects of iron metabolism. Thus, while studies have shown that all individuals in the 26 well-characterized families who had hereditary iron overload carried two copies of the C282Y mutation, not all those who were homozygous for the mutation developed iron overload. This obviously requires further study. The question of phenotypic variation is not resolved but the identification of the putative gene may assist in unraveling this problem. The reports that approximately 20% of homozygous subjects may not express the disease complicate the issue of diagnostic testing, and the appropriateness of genetic testing in contrast to the use of phenotypic markers such as transferrin saturation must be further addressed.

#### The Association of the Putative Hemochromatosis Gene with Other Conditions Related to Iron Metabolism

##### Porphyria cutanea tarda

A number of studies have previously been carried out 38-40 to examine the possibility that heterozygosity for haemochromatosis contributes to the disease porphyria cutanea tarda (PCT). This condition is a disorder of porphyrin metabolism associated with decreased activity of uroporphyrinogen decarboxylase (URO-D) within the liver. Liver damage of varying severity is frequent and PCT has been associated with alcohol abuse, iron overload, and infection with hepatitis C. It is also known that reduction of iron stores by phlebotomy frequently induces clinical and biochemical remission. Although previous studies have suggested that heterozygosity for genetic hemochromatosis is a common cause for the slightly increased iron stores in all these disorders,<sup>38,39</sup> Beaumont et al.<sup>40</sup> found no difference in the frequency of HLA-A3 in patients with sporadic PCT, familial PCT, and normal controls. Roberts et al.<sup>41</sup> have recently reported a significant increase in the frequency of the C282Y mutation in Welsh patients

with sporadic PCT compared with controls and a further study in Australia (Stuart et al, personal communication) has indicated that the frequency of the mutation in PCT patients is significantly greater than the frequency in the general population. Although patients who were shown to be heterozygous for the mutation showed no difference in transferrin saturation or serum ferritin concentration from those PCT patients with no mutation, both populations had a significantly higher transferrin saturation than normal healthy populations. Thus, it seems likely that factors other than the C282Y mutation are responsible for altered indices of iron metabolism in PCT.

The discovery of this gene has important clinical implications and the possible role of a malfunction in such a gene in refractory iron-deficiency states awaits further investigation. But perhaps the most fascinating consequence of its identification remains the possibility that some of the enigmas of iron metabolism in normal subjects, all of which have defied previous attempts at elucidation, may soon be explained.

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#### Section Description

This volume of Nutrition Reviews is also a supplement of the Scandinavian Journal of Nutrition/N&#228;ringsforskning, Volume 1, 1998. Supplement no 33:ISSN 1102-6510

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Accession Number: 00006226-199802020-00006  
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